Tampere University

KALLE KAAPU

Antiarrythmic Drugs and Cancer In Finnish Men

An epidemiological study on prostate cancer risk, survival and overall cancer mortality

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ACADEMIC DISSERTATION
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the Faculty of Medicine and Health Technology
of Tampere University,
for public discussion in the Paavo Koli Auditorium
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Dedication

To each and every one who supported me with this project.

ABBREVIATIONS

5-ARI 5-alpha-reductase inhibitors
ACE Angiotensin converting enzyme
ADT Androgen-deprivation therapy
ATBC The α-Tocopherol, β-Carotene Cancer Prevention Study
ATRB Angiotensin receptor blocker
AUC Area under the curve
BB Beta-blocker
BPH Benign prostatic hyperplasia
CCB Calsium channel blocker
CCI Charlson co-morbidity index
CCP Cell cycle progression
CI Confidence interval
DDD Defined daily dose
DRE Digital rectal examination
EAU European Association of Urology
EORTC The European Organization for Research and Treatment of Cancer
ER Estrogen receptor
ERSPC European Randomized study of Screening for Prostate Cancer
FinRSPC Finnish Randomized Study of Screening for Prostate Cancer
GG Grade Grouping
GnRH Gonadotropin-releasing hormone
GRS Genetic risk score
HIF-1 Hypoxia-inducible factor 1
HR Hazard ratio
ICD-10 International Classification of Diseases
ISUP International Society of Urologic Pathology
LDL Low-density lipoprotein
mpMRI Multiparametric magnetic resonance imaging
MRI Magnetic resonance imaging
NCI National Cancer Institute

NSAID Non-steroidal anti-inflammatory drugs
OR Odds ratio
PCPT Prostate Cancer Prevention Trial
PHI Prostate Health Index
PLCO Prostate, Lung, Colorectal, and Ovarian Cancer Screening Trial
PTEN Phosphatase and Tensin homolog
PSA Prostate-specific antigen
RALP Robotic-assisted laparoscopic prostatectomy
RANKL Receptor activator of nuclear factor-kB ligand
RCT Randomized controlled trial
RR Rate Ratio
TRT Testosterone replacement therapy
SII Social Insurance Institution of Finland
SNP Single nucleotide polymorphism
TRUS Transrectal ultrasound
WHO World Health Organization

ABSTRACT

Prostate cancer is the most frequent cancer and the second most common cause of cancer death among men in Europe. Since prostate cancer is a common disease and tumours usually grow slowly, multiple agents have been researched for chemoprevention of prostate cancer. Digoxin has been suggested to be a promising chemopreventive agent since *in vitro* studies have provided encouraging results. We used information from Finnish national health care registries and the Finnish Randomized Study of Screening for Prostate Cancer (FinRSPC) to evaluate potential associations between digoxin or other antiarrhythmic drug use and prostate cancer risk, prostate cancer -specific survival and overall cancer mortality.

Two large study populations were used. The case-control study included all new prostate cancers diagnosed in Finland during 1995-2002. Controls individually matched by age and area of residence at the time of the diagnosis were identified from the Population Register Center of Finland. Finally, a total of 24,657 case-control pairs were included in the study. The other study population contained 80,458 men participating in the FinRSPC, in which, 31,866 men were invited to prostate-specific antigen (PSA) screening. The rest of the study population formed the control arm and received no intervention. We obtained information on reimbursed antiarrhythmic medication purchases from the national prescription database of the Social Insurance Institution of Finland (SII).

Compared to non-users of antiarrhythmic drugs, digoxin users had a similar risk of prostate cancer and advanced prostate cancer both in the case-control study and the cohort study. We observed, however, a decreasing trend in the risk of Gleason 7-10 prostate cancer by duration of digoxin use in the cohort study, suggesting that long-term use of digoxin might decrease the risk. Other antiarrhythmic drug use was not associated with prostate cancer risk, with the exception of a diminished risk of advanced prostate cancer in the case-control study. However, sotalol use was associated with neither overall prostate cancer risk nor advanced prostate cancer risk in the cohort study. In the analysis of prostate cancer survival, digoxin use was not associated with the risk of prostate cancer death. Similar results were found for sotalol use and any antiarrhythmic drug use. In the cancer mortality study, digoxin users had increased overall risk of cancer death compared to non-users. Similarly,

sotalol use and any antiarrhythmic drug use was associated with increased cancer mortality. However, background co-morbidities modified the risk associations and long-term use of antiarrhythmic medication was not associated with an increased cancer mortality. Therefore, the association between antiarrhythmic drug use and cancer death is likely non-causal.

In conclusion, we found that use of digoxin and other antiarrhythmic drugs does not increase prostate cancer risk, reduce prostate cancer survival or increase cancer mortality. Our results and previous studies suggest that long-term use of digoxin might reduce prostate cancer risk, but such an effect is probably weak, since it has not been observed among our large study populations.

TIIVISTELMÄ

Eturauhassyöpä on länsimaiden yleisin syöpä ja toiseksi yleisin syöpäkuoleman aiheuttaja miehillä. Koska eturauhassyöpä on yleinen tauti ja kasvain kasvaa yleensä hitaasti, useita mahdollisesti syövän kehittymistä inhiboivia lääkeaineita on tutkittu. Digoksiinin on ehdotettu olevan varteenotettava eturauhassyövä kasvua ehkäisevä lääkeaine, sillä *in vitro* -tutkimukset ovat olleet lupaavia. Selvitimme kansallisten rekisterien ja suomalaisen eturauhassyövän seulontatutkimuksen (FinRSPC) aineiston avulla digoksiinin ja muiden rytmihäiriölääkkeiden käytön yhteyttä eturauhassyövän riskiin, ennusteeseen sekä yleiseen syöpäkuolleisuuteen.

Tutkimuksessa käytettiin kahta suurta aineistoa. Tapaus-verrokkitutkimuksessa aineiston muodostivat kaikki Suomessa diagnosoidut uudet eturauhassyöpätapaukset vuosina 1995-2002 sekä näille iän ja asuinalueen perusteella väestörekisteristä valitut verrokit. Lopullisen tutkimusaineston muodostivat 24,657 yksittäin valittua tapaus-verrokki -paria. Toisen tutkimusaineston muodostivat 80,458 FinRSPC:hen osallistunutta miestä. Seulontatutkimuksessa 31,866 miestä kutsuttiin prostataspesifisen antigeenin (PSA) mittaukseen. Loput aineistosta kuuluivat kontrolliryhmään, eikä heihin kohdistettu interventioita. Tieto rytmihäiriölääkeostoista poimittiin Kansaneläkelaitoksen reseptitietokannasta.

Digoksiinin käyttö ei ollut yhteydessä eturauhassyövän tai levinneen eturauhassyövän riskiin, kun vertasimme digoksiinin käyttäjiä miehiin, jotka eivät käyttäneet rytmihäiriölääkkeitä. Tulokset olivat samankaltaiset sekä tapaus-verrokkitutkimuksessa että kohorttitutkimuksessa. Kohorttitutkimuksessa havaittiin kuitenkin Gleason 7-10 eturauhassyövän riskissä laskeva trendi suhteessa digoksiinin käyttöaikaan, mikä viittaa siihen, että pitkäaikainen digoksiinin käyttö saattaa pienentää aggressiivisen eturauhassyövän riskiä. Muiden rytmihäiriölääkkeiden käyttö ei ollut yhteydessä eturauhassyöpäriskiin lukuun ottamatta sotalolia, jonka käyttäjillä oli matalampi levinneen eturauhassyövän riski tapaus-verrokkitutkimuksessa. Sotalolin käyttäjien eturauhassyöpäriski tai levinneen eturauhassyövän riski eivät kuitenkaan poikenneet ei-käyttäjien riskeistä kohorttitutkimuksessa. Digoksiinin käyttö ei ollut yhteydessä eturauhassyöpäpotilaan elossaoloaikaan. Vastaavat tulokset havaittiin myös sotalolin käytölle ja rytmihäiriölääkkeiden käytölle kaiken kaikkiaan. Viimeisessä osatyössä

havaittiin, että digoksiinin käyttäjillä oli korkeampi syöpäkuolleisuus kuin ei-käyttäjillä. Myös sotalolin käyttäjillä sekä rytmihäiriölääkkeiden käyttäjillä kaiken kaikkiaan oli korkeampi syöpäkuoleman riski kuin ei-käyttäjillä. Liitännäissairaudet muovasivat havaittua yhteyttä ja pitkäaikainen rytmihäiriölääkkeiden käyttö ei ollut yhteydessä suurentuneeseen syöpäkuolleisuuteen. Näin ollen rytmihäiriölääkkeiden ja suurentuneen syöpäkuolleisuuden välillä ei todennäköisesti ole syy-seuraussuhdetta.

Digoksiinin tai muiden rytmihäiriölääkkeiden käyttö ei suurenna eturauhassyöpäriskiä tai syöpäkuolleisuutta, ja on näin ollen turvallista. Tuloksemme yhdessä muiden tutkimusten kanssa viittaa siihen, että pitkäaikainen digoksiinin käyttö saattaa alentaa eturauhassyöpäriskiä, mutta vaikutus on todennäköisesti vähäinen, sillä se ei tule esiin suurissakaan aineistoissamme.

ORIGINAL PUBLICATIONS

Kaapu KJ, Ahti J, Tammela TLJ, Auvinen A, Murtola TJ. 2015. Sotalol, but not digoxin is associated with decreased prostate cancer risk: A population-based case-control study. International Journal of Cancer. 137(5):1187-95.

Kaapu KJ, Murtola TJ, Määttänen L, Talala K, Taari K, Tammela TLJ, Auvinen A. 2016. Prostate cancer risk among users of digoxin and other antiarrhythmic drugs in the finnish prostate cancer screening trial. Cancer Causes & Control. 27(2):157-64.

Kaapu KJ, Murtola TJ, Talala K, Taari K, Tammela TLJ, Auvinen A. 2016. Digoxin and prostate cancer survival in the Finnish Randomized Study of Screening for Prostate Cancer. British Journal of Cancer. 115(11):1289-95.

Kaapu KJ, Rantaniemi L, Talala K, Taari K, Tammela TLJ, Auvinen A, Murtola TJ. 2018. Cancer mortality does not differ by antiarrhythmic drug use: A population-based cohort of Finnish men. Scientific Reports. 9;8(1):10308.

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1 INTRODUCTION

Prostate cancer is the most common cancer among men in the Western World including in Finland and the second most frequent cancer after lung cancer among men worldwide (Ferlay et al. 2015). Prostate cancer usually has a good prognosis. 5-year survival rate for prostate cancers diagnosed between 2010-2014 was at least 90% in 25 countries and 80-89% in 17 countries (Allemani et al. 2018). However, globally approximately 307,000 men died due to prostate cancer in 2012 (Ferlay et al. 2015; Noone et al. 2017). The etiology of prostate cancer is incompletely understood but probably prostate cancer is generated from damaged prostate epithelium and progresses along decades (Rosenberg et al. 2010).

Due to long disease progression, several agents have been researched for prostate cancer chemoprevention. It has been observed that 5-alpha- reductase inhibitors (5-ARI) reduce prostate cancer risk (Andriole et al. 2010; I. M. Thompson et al. 2003). In addition, statins have been found to potentially have antineoplastic properties and decrease risk of prostate cancer (Jespersen et al. 2014; Kantor et al. 2015; Murtola et al. 2010).

Digoxin seems to be a promising antineoplastic agent. Even though *in vitro* experiments have been encouraging, results of observational studies have been conflicting (Flahavan et al. 2014; Kao et al. 2018; Platz et al. 2011; Zhang et al. 2008). We studied prostate cancer risk among users of digoxin and other antiarrhythmic drugs in two large study population: the first included all new prostate cancer cases in Finland during 1995-2002 and matched controls. The other consisted of men included in the Finnish Randomized Study of Screening for Prostate Cancer (FinRSPC). Furthermore, we evaluated the prostate cancer-specific survival and overall cancer mortality among antiarrhythmic drugs users participating in the FinRSPC trial.

2 REVIEW OF THE LITERATURE

2.1 Prostate cancer definition and diagnosis

Prostate is an exocrine gland of the male reproductive system and it consists of glandular cells, myoepithelial cells and subepithelial interstitial cells. In most cases, prostate cancer originates from glandular cells in the peripheral zone of the prostate and can therefore be classified as an adenocarcinoma. Rarely, prostate cancer can arise from epithelial origin. Carcinogenesis is a complex process including activation of several oncogenes and deactivation of tumor suppression genes. Some of these mutations are known, TMPRSS2:ERG gene fusion and Phosphatase and Tensin homolog (PTEN) deletion, for example (Jamaspishvili et al. 2018; Z. Wang et al. 2017). The development from carcinoma in situ to clinically detectable cancer lasts usually at least several years. If the tumor is aggressive, it is likely to spread first to the pelvic lymph nodes and afterwards to the skeleton, most typically vertebrae, ribs and pelvis (Bubendorf et al. 2000).

2.1.1 Detection

Early prostate cancer is nearly invariably asymptomatic. Classic clinical symptoms of prostate cancer are due to urinary obstruction and resemble those of benign prostate hyperplasia (BPH). The most common symptoms are frequent urination, difficulties in maintaining adequate urine flow, urinary obstruction, nocturia, dysuria and hematuria. Advanced prostate cancer can give systemic symptoms, such as unintentional weight loss, fever, anemia, fatigue and bone pain (typically in spine) or fractures (Taari et al. 2013).

The first clinical exam is the digital rectal examination (DRE). Typical findings in prostate cancer include abnormally hard or irregular prostate. It is important to consider that early-stage tumor might not be palpable and therefore to detect early-stage cancers further examination is required (Duodecim 2014).

PSA concentration helps clinicians to decide which patients might benefit from additional urological examination. Patients with evidently high PSA levels should be

referred to a urologist, but often PSA level is only marginally over the reference levels (Taari et al. 2013). If total PSA is 2.5 - 10 ug/l, it is useful to determine the proportion of free PSA. Low free PSA concentration indicates an increased prostate cancer risk and 15 % is considered as a cut-point to decide whether a patient needs further examination (Duodecim 2014). Probability of prostate cancer at certain levels of PSA and free PSA percentages are presented in Table 1. It is essential to comprehend that poor sensitivity is the most relevant disadvantage of PSA testing. There is a lot of variation in sensitivity and specificity of PSA depending on a study population and a method used to confirm the prostate cancer diagnosis. The American Cancer Society concluded that baseline PSA of 4.0 ug/l or more has a sensitivity of 21% and specificity of 91% (Wolf et al. 2010).

In suspicious but unclear cases it is important to monitor PSA concentration since with prostate cancer PSA level usually rises over time. PSA velocity over 0.75 ug per year is an indication for further examination. In addition, if 5-ARI has been prescribed for the patient, PSA level should decrease 50% during the treatment and if this does not occur, the possibility of prostate cancer should be excluded.

Urologist's basic exams to patient with suspected prostate cancer are transrectal ultrasound (TRUS) and prostate biopsy. TRUS is useful to evaluate the size and consistency of the prostate, but malignancy cannot be excluded by TRUS. Prostate biopsy is conveniently taken after ultrasound. It is recommended to take 12 tissue samples at different parts of the prostate. Negative biopsy results do not definitively exclude a prostate cancer, so examination should be repeated if malignancy is clinically probable. (Duodecim 2014).

Magnetic resonance imaging (MRI) provides additional information besides classic diagnostic methods. The primary indication for MRI is a situation, in which prostate biopsy is negative but PSA increases during a follow-up. If a suspicious area is found, MRI-targeted prostate biopsies are taken. The cancer detection percentage among men with previous negative biopsies was found to be higher with MRI-targeted biopsy compared to TRUS-guided biopsy (46% vs. 23%, $p<0.05$) and cancers diagnosed with MRI-targeted biopsy showed more features of clinical significance (biopsy Gleason pattern ≥ 4 or tertiary pattern 5, serum PSA >10 ug/l and PSA density >0.15 ug/l/cm3) (Kaufmann et al. 2015). In the PRECISION trial, among the MRI-targeted biopsy group Gleason score 3+4 or greater cancer was detected in 95 men (38%) whereas among men in the standard-biopsy group clinically significant cancer was detected in 64 men (26%) ($p = 0.005$) (Kasivisvanathan et al. 2018). However, conflicting results have been published (Arsov et al. 2015). In the future, it might be possible to perform MRI and take

targeted biopsies before or instead random prostate biopsies. Studies have observed that the method mentioned above reduces the detection of low-grade prostate cancers and the number of biopsies whereas the detection of clinically significant prostate cancer is improved (Delongchamps et al. 2013; Garcia Bennett et al. 2017; Pokorny et al. 2014).

Due to poor sensitivity of PSA, several new prostate cancer detectors are being researched. A four-kalligrein panel called 4Kscore includes total PSA, free PSA, intact PSA and kallikrein-related peptidase 2. Combining markers mentioned above can reduce unnecessary prostate biopsies. Data from ERSPC shows that four-kallikrein panel had better predictive accuracy compared to PSA and age alone (the area under the curve (AUC) of 0.711 vs 0.585, $p<0.001$) (Vickers et al. 2010). The Stockholm 3 model is a combination of plasma protein biomarkers (PSA, free PSA, intact PSA, hK2, MSMB, MIC1), gene polymorphisms (232 Single nucleotide polymorphisms (SNPs)), and clinical variables (age, family history, previous prostate biopsy). When the Stockholm 3 model was compared to PSA testing only, the Stockholm 3 was significantly better for detection of prostate cancers with a Gleason score 7 or more (the AUC 0.74, 95% CI 0.72-0.75 vs 0.56, 95% CI 0.55-0.60, $p<0.0001$) (Scott et al. 2017).

The Prostate Health Index (phi) is a combination of three different isoforms of PSA: total PSA, free PSA, and [−2]proPSA. An Italian study of 268 men with PSA levels of 2-10 ng/ml and negative DRE evaluated phi. Men were referred to extended prostate biopsy with the primary objective to compare phi with commonly used tests, total PSA, free PSA percentage and PSA density. Diagnosed prostate cancer cases (39.9%) had a higher phi (median 44.3 compared to 33.1, $p < 0.001$). Phi had superior sensitivity (42.9%) than free PSA percentage (20.0%) or PSA density (26.5%) and predictive accuracy (AUC 0.76 for phi) than PSA density (AUC 0.61), free PSA percentage (AUC 0.58) or total PSA (AUC 0.53) (Guazzoni et al. 2011). A similar observation was made in a US study (Catalona et al. 2011).

In addition, biomarkers can be used to distinguish prostate cancer from BPH even though they are not commonly used. The Prolaris cell cycle progression (CCP) test is a gene test evaluating how quickly neoplastic cells proliferate. It has been suggested to be potentially used to advance the accuracy of individual risk evaluation. However, a review evaluating two before-after studies observed that even though CCP test may change the treatment for some low- and intermediate-risk patients it would result in a major increase in cost to the health care budget (Health Quality Ontario 2017).

Table 1. Likelihood of prostate cancer at certain PSA (prostate specific antigen) concentrations (Jousimaa et al. 2017).

Total PSA	Probability of prostate cancer
0-2 ug/l	1%
2-4 ug/l	15%
4-10 ug/l	25%
>10 ug/l	>50%
Free PSA percentage when total PSA between 4-10 ug/l	
0-10%	56%
10-15%	28%
15-20%	20%
20-25%	16%
>25%	8%

2.1.2 Screening

Two large screening trials commenced in the 1990s to evaluate whether PSA screening can reduce prostate cancer mortality; the European Randomized Study of Screening for Prostate Cancer (ERSPC) (Schröder et al. 2014) and the Prostate, Lung, Colorectal and Ovarian (PLCO) Cancer Screening Trial by the U.S. National Cancer Institute (NCI) (Pinsky et al. 2017).

The main results differed slightly. PSA screening reduced prostate cancer-specific mortality (RR (Rate ratio) 0.80, 95% CI (Confidence interval) 0.72-0.89) at 16 years of follow-up in the ERSPC (Hugosson et al.). However, there was no difference

between the screening arm and the control arm (RR 1.04, 95% CI 0.87-1.24) in the PLCO Cancer Screening Trial (Pinsky et al. 2017). The difference in results between these two large trials might be explained by more common PSA testing prior to randomization and contamination testing in the PLCO control arm during the trial since the PLCO study population consisted of American citizens and a random PSA testing was more common in America than in Europe during that era. Furthermore, biopsy compliance was only 25% approximately in the PLCO study (Schröder and Roobol 2010). When differences (enrollment and attendance patterns, screening intervals, PSA thresholds, biopsy receipt, control arm contamination, and primary treatment) in the ERSPC and the PLCO studies were taken into account, the PLCO study provides consistent evidence that PSA screening might decrease prostate cancer mortality (de Koning et al. 2018; Tsodikov et al. 2017).

PSA screening has multiple adverse effects. Overdiagnosis is probably the most severe hindrance and it concerns especially clinically insignificant low-grade cancers. A second problem is lead time, which means time that screening advances cancer diagnosis. Draisma et al. 2003 evaluated effects of lead time and overdetection among the ERSPC study population. Authors concluded that mean lead times and overdetection rate depended on age of patients at screening. Mean lead time was calculated to be 12.3 years and the overdetection rate was 27% for a single screening test at age 55. However, mean lead time was 6.0 years and the overdetection rate 56% at age 75 (Draisma et al. 2003). Prostate cancer incidence was higher in the screening arm compared to the control arm in both studies (RR 1.57, 95% CI 1.51-1.62 in the ERSPC and RR 1.27, 95% CI 1.20-1.35 in the PLCO). In the ERSPC, one prostate cancer death was avoided per 781 screening invitation and per 27 additional prostate cancer diagnoses. A prostate biopsy is an invasive operation and approximately 1% of men undergoing it ended up with a severe adverse effect, infection for example (Chou et al. 2011). Minor complications are common; a study evaluating 5957 prostate biopsies reported that hematospermia occurred after 36.3% of biopsies, hematuria after 14.5% and rectal bleeding persisting for up to 2 days after 2.3% (Berger et al. 2004).

Overdiagnosis leads to overtreatment and prostate cancer treatment options have difficult adverse effects. Radical prostatectomy was associated with erectile dysfunction (14.6% vs 5.4%) and incontinence (17.3% vs 4.4%) compared to the control group (Wilt et al. 2017). Men receiving external-beam radiation therapy had comparable adverse effects with men with radical prostatectomy after 15 years follow-up (Resnick et al. 2013).

In Finland, prostate cancer incidence has increased strongly alongside generalized PSA testing. However, prostate cancer prognosis has improved concurrently. Age-adjusted 5-year survival was 41.96% among prostate cancer cases diagnosed during 1969-1971 whereas for prostate cancer cases diagnosed between 2011-2013 5-year survival was 91.98% (Finnish Cancer Registry a).

One of the future goals is to find men benefiting the most from PSA screening. Epidemiological studies have shown that men with PSA concentration below median have minimal risk of advanced prostate cancer during the next 15 years (0.28%, 95% CI 0.11-0.66) (Vickers et al. 2013). Therefore, PSA screening should be focused on men with high PSA concentration at the baseline. Further screening for men with PSA $\geq$ 1.0 ug/l at age of 40 years and $\geq$ 2.0 ug/l at age of 60 years might be reasonable (Carlsson et al. 2014; Vickers et al. 2013).

New prostate cancer screening methods are currently being researched. The ProScreen trial started in 2018 and it involves new procedures to detect clinically relevant prostate cancers. Study population will consist of 67,000 men aged between 50-63 years at the start of the follow-up. A quarter of the study population will be allocated to the screening arm and the rest will form the control arm, which will receive no intervention. The screening arm participants will be invited to a PSA test and men with PSA of 3 ug/l or higher will receive a further multi-kallikrein panel. Multiparametric magnetic resonance imaging (MpMRI) will be performed to patients with a risk of clinically significant prostate cancer >7.5% and finally, men with a suspect finding in MRI are directed to targeted biopsies. The objective is to reduce overdiagnosis without losing mortality benefit (Auvinen et al. 2017).

2.1.3 The Gleason grading system

The Gleason grading system is an important part of evaluation of prostate cancer prognosis (Gleason and Mellinger 1974). A pathologist evaluates tissue samples and gives a Gleason grade based on its glandular architecture. A grade can vary between 1 to 5, a lower score representing less aggressive pattern. Two grades are given – the first one is based upon the predominant pattern and a second grade on the second most common pattern. The sum of the two grades gives the Gleason score. Figure 1 demonstrates Gleason grading system (N. Chen and Zhou 2016).

When 305 men with prostate cancer were followed, the disease-specific survival for Gleason score 4-5 was 20 years, for Gleason score 6 survival was 16 years, for score 7 10 years and for score 8-10 5 years ($p < 0.001$) (Egevad et al. 2002). When

biochemical recurrence-free survival among men with different Gleason scores were studied, clear trend was observed. Five-year survivals were 94.6% for men with Gleason score ≤ 6, 82.7% for score 3 + 4, 65.1% for score 4 + 3, 63.1% for score 8 and 34.5% for score 9 – 10 (Pierorazio et al. 2013).

The International Society of Urological Pathology (ISUP) published a consensus in 2005 proposing a new modification of the Gleason score called Grade Grouping (GG). If Gleason score is Gleason ≤ 6, GG is 1, Gleason score 3+4 means GG 2, Gleason score 4+3 forms GG 3, Gleason score 8 (4+4, 3+5, 5+3) means GG 4 and Gleason score 9-10 (4+5, 5+4, 5+5) form GG 5. GG 1 cancer is a low-risk disease, GG 2-3 are intermediate-risk cancers and GG 4-5 are high-risk cancers (Epstein et al. 2016).

However, the Gleason grading system has some limitations. Gleason score depends on location of a prostate tissue sample since there might be a lot of histological heterogeneity within a tumor, and it is always a subjective estimate of a pathologist.

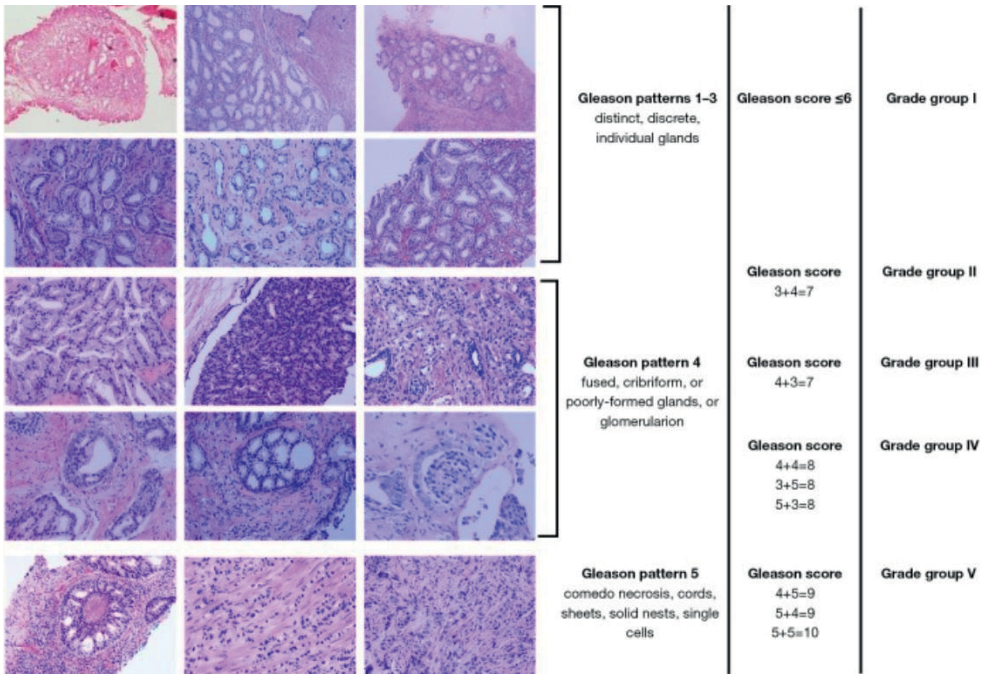

Figure 1. Gleason grading system and typical Gleason patterns. Reused with permission from AME Publishing Company (N. Chen and Zhou 2016).

2.1.4 TNM classification and staging

The TNM classification of Malignant Tumors is commonly used system for staging cancer (O'Sullivan et al. 2015). It consists of three individual parts. T describes size or local extension of the primary tumor. N describes invasion to regional lymph nodes and M describes extent of metastases. TNM classification can be divided in two separate systems: clinical and pathological TNMs. cTNM is determined clinically based on DRE, prostate biopsy and further imagining such as prostate MRI and bone scan. pTNM is microscopically determined by pathologist after surgery, such as radical prostatectomy. N-stage is possible to determine reliably only after lymphadenectomy. TNM classification for prostate cancer is described in Table 2 (Cheng et al. 2012). TNM classification can be used to classify prostate cancer on different stages and stage grouping is described in Table 3 (Cheng et al. 2012). Stage grouping can be further used to classify cancer to local, locally advanced or advanced disease. Local disease includes stages I and II, locally advanced disease means stages III and IV (excluding M1 disease) and M1 forms advanced disease.

TNM classification influences treatment decision. This is further discussed in the Treatment-chapter.

Table 2. TNM staging for prostate cancer (Cheng et al. 2012)

Stage	Properties
TX	Primary tumor cannot be assessed
T0	No evidence of primary tumor
T1	Clinically inapparent tumor neither palpable nor visible by imaging
T1a	Tumor incidental histological finding in ≤5% of tissue resected
T1b	Tumor incidental histological finding in >5% of tissue resected
T1c	Tumor identified by needle biopsy
T2	Tumor confined within prostate
T2a	Tumor involves ≤one-half of one lobe
T2b	Tumor involves >one-half of one lobe but not both lobes
T2c	Tumor involves both lobes
T3	Tumor extends through the prostate capsule
T3a	Extracapsular extension (unilateral or bilateral)
T3b	Tumor invades seminal vesicle(s)
T4	Tumor is fixed or invades adjacent structures other than seminal vesicles such as external sphincter, rectum, bladder, levator muscles, and/or pelvic wall
NX	Regional lymph nodes were not assessed
N0	No regional lymph node metastasis
N1	Metastases in regional lymph node(s)
M0	No distant metastasis
M1	Distant metastasis
M1a	Non-regional lymph node(s)
M1b	Bone(s)
M1c	Other site(s) with or without bone disease

Table 3. Stage grouping of prostate cancer by American Joint Committee on Cancer (Cheng et al. 2012).

Stage	T	N	M	PSA (ug/l)	Gleason Score
I	T1a-c	N0	M0	<10	≤6
	T2a	N0	M0	<10	≤6
	T1-2a	N0	M0	X	X
IIA	T1a-c	N0	M0	<20	7
	T1a-c	N0	M0	≥10 and <20	≤6
	T2a	N0	M0	<20	7
	T2b	N0	M0	<20	≤7
	T2b	N0	M0	X	X
IIB	T2c	N0	M0	Any PSA	Any Gleason
	T1-2	N0	M0	≥20	Any Gleason
	T1-2	N0	M0	Any PSA	≥8
III	T3a-b	N0	M0	Any PSA	Any Gleason
IV	T4	N0	M0	Any PSA	Any Gleason
	Any T	N1	M0	Any PSA	Any Gleason
	Any T	Any N	M1	Any PSA	Any Gleason

PSA = Prostate-specific antigen

2.2 Prostate cancer occurrence

2.2.1 Incidence and prevalence

Prostate cancer is the second most commonly diagnosed cancer among men globally. It has been estimated that there were 1,276,100 new prostate cancer cases in 2018. The cumulative risk of prostate cancer to age 75 was 3.73% and age standardized rate was 29.3/100,000 person-years worldwide (Ferlay et al. 2019). Incidence of prostate cancer is higher in more developed regions (758,700 new cancer cases in 2012, 12.5 % of all cancer cases) than less developed regions (353,000 new cancer cases in 2012, 4.4 % of all cancer cases). Age-standardized rates of prostate cancer incidence were 69.5/100,000 person years and 14.5/100,000 person years in more and less developed countries, respectively. (Ferlay et al. 2015)

In Finland, the Finnish Cancer Registry compiles statistics of all Finnish cancer cases. A total of 5,162 new prostate cancer cases were diagnosed in 2016 (Finnish Cancer Registry b) and the age standardized incidence rate (global standard population) was 82.5/100,000 person-years (Finnish Cancer Registry c). At the beginning of 2015, 36,357 prostate cancer patients (prostate cancer diagnosis within less than 10 years) were alive in Finland (Finnish Cancer Registry d) and the prevalence was 1,340/100,000 (Finnish Cancer Registry e).

Autopsy studies have suggested that prostate cancer is a common incidental finding. Among men aged between 70-79 years, the prevalence of prostate cancer was 50.5% in U.S. blacks, 35.7% in U.S. whites and Europeans, and 21.2% in Asians and prevalence of prostate cancer increased with every decade of age (OR 1.7, 95% CI 1.6-1.8) (Bell et al. 2015; Jahn, Giovannucci, Stampfer 2015).

2.2.2 Mortality and prognosis

Prostate cancer is a major cause of death worldwide but especially in North America, Europe and Oceania. Approximately 307,500 men died from prostate cancer worldwide in 2012. Age-standardized prostate cancer mortality was 10.0/100,000 person-years in more developed countries, notably higher compared to 6.6/100,000

person-years in less developed countries. (Ferlay et al. 2015). Ten-year relative survival has increased radically over the decades: it was 53.2% for prostate cancers diagnosed in 1975-1979 whereas in 2005 the rate was 99.2% in the U.S. (Howlader et al. 2016).

In 2016, there were 900 prostate cancer deaths in Finland. Age-standardized mortality rate (global standard population) was 11.38/100,000 person-years (Finnish Cancer Registry a). Five year age-standardized survival among men aged 15–99 years diagnosed with prostate cancer during 2010-2014 was 93.2% in Finland, 90.7% in Sweden, 97.4% in U.S., 94.5% in Australia, 79.3% in Russian Federation, 69.2% in China and 58.7% in Nigeria (Allemani et al. 2018).

Prostate cancer prognosis has improved over the past decades, as shown in Table 4, due to more developed cancer treatments and earlier detection (Howlader et al. 2016). Before the PSA era majority of prostate cancer patients died due to the disease. Alongside with early detection, prognosis have improved radically. However, prognosis depends considerably on stage and grade of the disease (Mottet et al. 2017). Despite improved treatment, prognosis for advanced prostate cancer remains low. Five-year survival percent by stage at diagnosis is presented in Table 5 (Finnish Cancer Registry b).

The European Association of Urology has composed prognostic risk groups by PSA, TNM stage and Gleason score. Tumor is defined as a low-risk disease if PSA is <10 ug/l, Gleason score is <7 and T-stage is T1-2a. Intermediate-risk disease exists if PSA is between 10-20 ug/l, Gleason score is 7 or T-stage is 2b. If PSA is over 20 ug/l, Gleason score is more than 7 or T-stage is T2c there is a high-risk local disease (Mottet et al. 2017).

Table 4. 5-year relative survival 2007-2013 by stage at diagnosis. Data from U.S. National Cancer Institute (Howlader et al. 2016).

Stage at diagnosis	Survival percent
All stages	98.6
Localized	100.0
Locally advanced	100.0
Advanced	29.8
Unstaged	81.2

Table 5. 5-year relative survival by year of diagnosis. Data from Finnish Cancer Registry (Finnish Cancer Registry e).

Year of diagnosis	Survival percent
1975-1977	55.0
1978-1980	55.0
1981-1983	59.9
1984-1986	55.8
1987-1989	62.8
1990-1992	61.8
1993-1995	65.6
1996-1998	73.3
1999-2001	81.1
2002-2004	86.5
2005-2007	91.8
2008-2010	93.2
2011-2013	92.5
2014-2016	93.1

2.3 Prostate cancer etiology

2.3.1 Age

Age is the most important risk factor for developing prostate cancer. Even though prostate cancer is the most frequent cancer among men in the western world, its prevalence among men younger than 50 years is diminutive. Figure 2 demonstrates distribution of prostate cancer by age in Finland. The prostate gland requires androgens (testosterone, dehydroepiandrosterone and dihydrotestosterone) and especially dihydrotestosterone has been linked with prostate cancer progression. (Lonergan and Tindall 2011). Accumulated androgen burden might be one possible explanation for activation of oncogenes in the prostate.

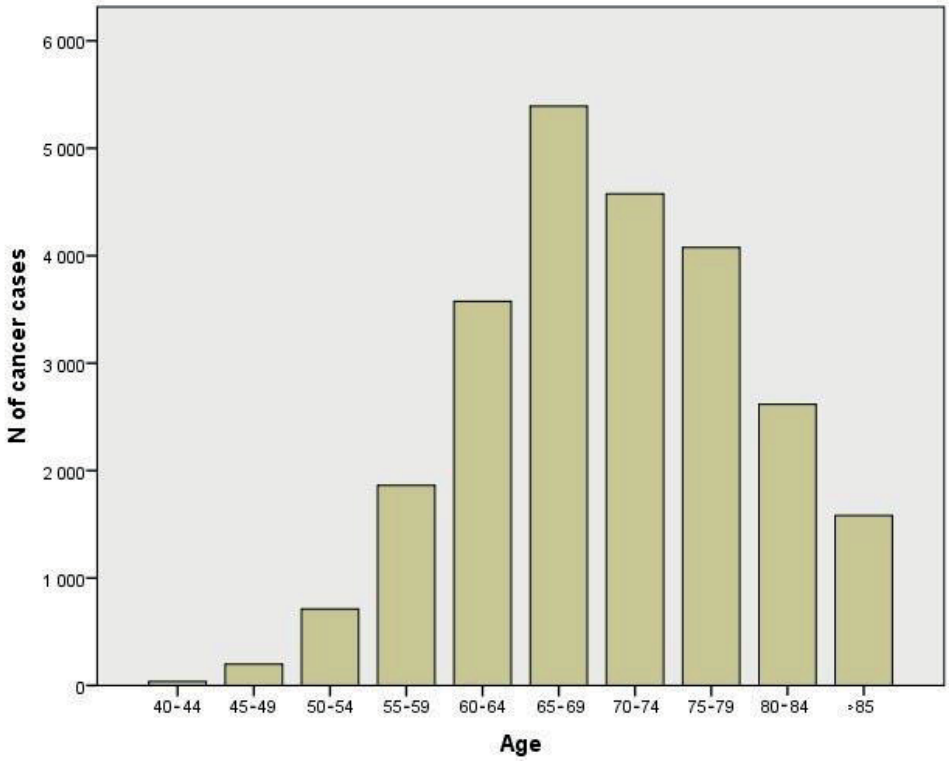

Figure 2. Number of new prostate cancer cases diagnosed among different age groups in Finland in 2012-2016 (Finnish Cancer Registry b).

2.3.2 Geography

The prostate cancer incidence varies considerably worldwide. The world age-standardized incidence rates are highest in Australia and New Zealand (86.4/100,000 person-years), whereas in South Central Asia the incidence rate is only 5.0/100,000 person-years. In semi-industrial world, the incidence rates are between these two extremes, for example, 60.4/100,000 and 42.2/100,000 person-years in South America and in Eastern Europe, respectively (Bray et al. 2018).

The substantially higher incidence rates in the industrial world likely reflect in part more active PSA screening and subsequent biopsies. Another explanation is a difference in burden of chronic diseases between high- and low-income countries. Chronic diseases might lead to underdiagnosis of almost asymptomatic prostate cancer.

Nevertheless, it has been reported that there is divergence by race in the prostate cancer incidence rate in the USA (Brawley 2012; Krieger et al. 1999; Siegel, Miller, Jemal 2016). The incidence rate is notably higher among black males (Incidence rates 208.7/100,000 person years) than among the Asians (67.8/100.000 person years) in the USA (Siegel et al. 2016). Various explanations have been provided for the racial disparities. Socioeconomic and behavioral factors might account but physiological, constitutional and genetic factors have an important role as well (Bhardwaj et al. 2017).

2.3.3 Genetic factors

Family history is a well-known risk factor for prostate cancer, and it has been studied extensively. Prostate cancer demonstrates Mendelian inheritance model and there is a rare high penetrant hereditary form (Carter et al. 1992; Pilie, Giri, Cooney 2016). Findings from Nordic twin registries have suggested that heritable factors have a greater effect for prostate cancer than any other cancers (Hjelmborg et al. 2014; Mucci et al. 2016). A review of 13 case-control and cohort studies estimated that the risk of prostate cancer is 2.5 times higher for men with first-degree relatives diagnosed with prostate cancer compared to the men without prostate cancer in the family. If there was more than one affected first-degree relative, the risk ratio increased to 3.5. The risk ratio was even higher (4.3 95% CI 2.9-6.3) for men with first-degree relatives diagnosed with prostate cancer before age of 60. (Johns and Houlston 2003).

Mutations in BRCA1/2 genes have been reported to have an association with increased prostate cancer risk (Ostrander and Udler 2008; D. Thompson, Easton, Breast Cancer Linkage Consortium 2002). However, several studies have shown that BRCA1/2 mutations have only minor influence on familiar prostate cancer risk since they are relatively rare (Agalliu et al. 2007; Ikonen et al. 2003; Sinclair et al. 2000). Nevertheless, a mutation named G84E in HOXB13 gene seems to have an association with hereditary prostate cancer (Ewing et al. 2012; Huang and Cai 2014; Laitinen et al. 2013).

Approximately 160 single nucleotide polymorphisms (SNPs) have been linked with increased prostate cancer risk. It has been estimated that the identified SNPs explain a third of the familial risk among European population. The RR for developing prostate cancer was 2.69 (95% CI 2.55-2.82) times higher among the top 10% of the men in the highest risk group and 5.71 (95% CI 5.04-6.48) times higher

among the top 1% of men in the highest risk group compared to the population average (Schumacher et al. 2018). The SNPs are concentrated at 12 regions and majority of the signals are related to known biological mechanisms including AR, ERG and FOXA1 (Dadaev et al. 2018).

In the PLCO trial, the study population was divided into groups by genetic risk score (GRS) and there was an association between GRS and prostate cancer detection rate (43.2%, 47.8%, 58.8% and 69.4% in the first, second, third and fourth quartiles, respectively, $p < 0.001$) (Liss et al. 2015). A similar observation was made with the population from the FinRSPC. The overdiagnosis percentage was 58% (95% CI 54–65) of the prostate cancers detected by PSA screening among men with the lower polygenic risk whereas men with higher polygenic risk had the overdiagnosis percentage of 37% (95% CI 31–47). 74% of all prostate cancers were diagnosed from men with polygenic risk over population median (Pashayan et al. 2015).

2.3.4 Behavioral risk factors

Besides many other cancers, smoking has been connected with fatal prostate cancer. Meta-analysis of 51 studies showed that smokers had significantly increased risk of prostate cancer death (RR 1.24, 95% CI 1.18-1.31). Surprisingly, current smoking was associated with decreased prostate cancer incidence (RR 0.90 95% CI 0.85-0.96) but this is probably explained by smoking promoting more aggressive cancers instead of prostate cancer. (Islami et al. 2014). Similar findings were obtained in two smaller reviews also (Huncharek et al. 2010; Zu and Giovannucci 2009).

A meta-analysis summarizing 22 randomized controlled trials (RCTs) assessed the association between testosterone replacement therapy (TRT) and prostate cancer. Neither short-term (< 12 months) nor long term (12-36 months) use of TRT increased risk of prostate cancer: odds ratio (OR) 0.39 (95% CI 0.06-2.45) for short-term and OR 2.09 (95% CI 0.18-24.73) for long-term use of testosterone injection treatment. Transdermal administration of TRT: OR was 1.10 (95% CI 0.26-4.65) for short-term use and 3.06 (95% CI 0.12-76.70) for long-term use. Current literature suggests that TRT does not increase risk of prostate cancer (Cui et al. 2014).

Two previous meta-analyses suggest that obesity is associated with both increased aggressive prostate cancer risk and decreased localized cancer risk (Discacciati, Orsini, Wolk 2012; Discacciati and Wolk 2014). The explanation for these opposite relationships between obesity and risk of localized and advanced prostate cancer

might be different concentrations of free testosterone in serum. Obese men tend to have lower testosterone concentration (Lima et al. 2000), which is a risk factor for aggressive prostate cancer (Platz et al. 2005b; Severi et al. 2006). Physical activity was associated with slightly reduced overall prostate cancer risk (RR 0.90, 95% CI 0.84-0.95) in a large meta-analysis when comparing men with the highest to men with the lowest level of activity (Y. Liu et al. 2011).

A large number of studies considering dietary factors and prostate cancer have been published. There is no solid association between reduced prostate cancer risk and any specific nutrient, but the most promising dietary factors for decreasing prostate cancer risk are the Mediterranean diet, soy protein, lycopene, vitamin E and green tea.

Adherence to the Mediterranean diet was associated with prolonged prostate cancer survival (HR for death 0.78, 95% CI 0.67-0.90) in the Health Professional Follow-up Study compared to low adherence (Kenfield et al. 2014) and a meta-analysis reported that men with the highest adherence to Mediterranean diet had slightly decreased prostate cancer risk compared to men with low adherence (RR 0.96, 95% CI 0.92-0.99) (Schwingshackl and Hoffmann 2014). There was significantly reduced prostate cancer risk among men consuming more soy food ($p < 0.001$), genistein ($p = 0.008$), daidzein ($p = 0.018$) and unfermented soy food ($p < 0.001$) in a previous review (Applegate et al. 2018). Both dietary consumption and circulating concentration of lycopene were associated with decreased prostate cancer risk (RR 0.88, 95% CI 0.78-0.98 and RR 0.88, 95% CI 0.79-0.98, respectively) in a large meta-analysis including 42 studies (Rowles et al. 2017). In the Finnish Alpha-Tocopherol, Beta-Carotene Cancer Prevention (ATBC) Study, vitamin E supplement use was connected with significantly decreased prostate cancer risk (RR 0.68, 95% CI 0.53-0.88) (Heinonen et al. 1998), but supplement use was directly associated with prostate cancer risk (RR 1.17, 95% CI 1.00-1.36) in the Selenium and Vitamin E Cancer Prevention Trial (Klein et al. 2011). A meta-analysis containing 38 studies observed decreased risk of prostate cancer among men with high selenium intake or plasma level compared to men with less (RR 0.86, 95% CI 0.78-0.94) (Sayehmiri et al. 2018). Men with the highest coffee intake had decreased risk of prostate cancer compared to men with the lowest intake (RR 0.90, 95% CI 0.85-0.95) in a meta-analysis of 13 cohort studies (H. Liu et al. 2015). Consumption of green tea reduced prostate cancer in a Chinese case-control study (OR 0.28, 95% CI 0.17-0.47) (Jian et al. 2004) and a similar finding was obtained in a Japanese prospective study (OR 0.52, 95% CI 0.28-0.96) (Kurahashi et al. 2007). Hackshaw-McGeagh et al. (2015) identified 44 RCTs of behavioral interventions with prostate

cancer progression or mortality outcomes. Only 10 of the trials were assessed as having good methodological quality and low risk of bias. Beneficial effects were observed in a trial of a nutritional supplement of pomegranate seed, green tea, broccoli, and turmeric, in a trial comparing flaxseed, low-fat diet, flaxseed, and low-fat diet versus usual diet and in a trial supplementing soy, lycopene, selenium, and coenzyme Q10 (Hackshaw-McGeagh et al. 2015).

Ejaculation frequency has been suggested to be associated with risk of prostate cancer. Men with increased sexual activity might have a reduced prostate cancer risk compared to men with less sexual activity. The Health Professionals' Follow-up Study observed that men with more than 20 ejaculation per month at ages 20–29 and 40–49 years had a decreased prostate cancer risk compared to men with average of 4–7 ejaculations per month (HR 0.81, 95% CI 0.72–0.92 and HR 0.78, 95% CI 0.69–0.89, respectively) (Rider et al. 2016).

2.4 Prostate cancer treatment

2.4.1 Active surveillance

Active surveillance is a reasonable alternative for first treatment of localized low-risk prostate cancer. It involves regular urologist appointments (at least annually), PSA tests (every 6 months) and repeated prostate biopsies (within the first year and then once every 3 to 5 years) according to European Association of Urology (EAU) guidelines. Since there are no prospective clinical trials comparing active surveillance to immediate surgical or radiotherapy treatment, selection criteria for active surveillance varies globally. In Finland, active surveillance is an option for men with Gleason score 6 or less, PSA less than 10 ug/l, T-stage less than T3 and 2 or less cores with cancer involvement in prostate biopsy (Duodecim 2014). If disease progresses during surveillance, switching to active treatment is indicated. Strongest indicators to start active treatment are Gleason score 7 or more, more than 2 cancer positive cores in prostate cancer biopsy or T-stage progression. PSA increase is less specific indicator compared to the previous ones (Dall'Era et al. 2012).

A Canadian prospective cohort study followed 993 men with low- or intermediate-risk prostate cancer. Overall and prostate cancer-specific survival rates after 15 years of follow-up were 62% and 94.8%, respectively. At 15 years, 55% of men were not treated but still on surveillance (Klotz et al. 2015).

The Prostate Testing for Cancer and Treatment study compared active surveillance (PSA test every three months for the first year and 1-2 times per year thereafter), radical prostatectomy and external-beam radiotherapy on 1643 men with localized prostate cancer. 17 prostate cancer-specific deaths occurred during the median follow-up of 10 years. 8 of them belong to the active surveillance group, 5 of them to the radical prostatectomy group and 4 of them to the radiotherapy group ($p=0.48$, for overall comparison) suggesting that treatment option does not have impact on the 10-year cancer-specific survival. 291 men (53%) among the active surveillance group received a radical treatment by the end of the follow-up. However, incidence of disease progression, including metastasis, was increased in the active surveillance group compared to the radical prostatectomy and radiotherapy groups. 112 men in the active surveillance group had disease progression, whereas 46 and 46 men had disease progression in the prostatectomy

and in the radiotherapy group, respectively ($p<0.001$, for the overall comparison). Since there was no difference in survival, it can be deduced that radical treatment after disease progression in active surveillance is safe (Hamdy et al. 2016).

2.4.2 Radical prostatectomy

Radical prostatectomy is a classic treatment option for localized prostate cancer. Surgically removing malignant prostate tissue improves survival compared to watchful waiting. Radical prostatectomy reduced both overall and cancer-specific mortality among men with localized prostate cancer in the SPCG-4 study (Bill-Axelson et al. 2011). There was no statistically significant difference between radical prostatectomy and watchful waiting in all-cause or cancer-specific mortality in the PIVOT trial. However, among men with PSA over 10 ug/l or with intermediate- or high-risk tumor all-cause mortality was increased in the control arm (Wilt et al. 2012). The SPCG-4 study started before common PSA testing era at 1989 so the study included a lot of advanced cancer cases. On the other hand, the PIVOT trial has been conducted during the PSA era and it includes early stage tumors. This divergence in study populations probably explains the difference in the outcomes.

The most common side-effects of radical prostatectomy are incontinence and sexual dysfunction. Long-term urine incontinence and sexual dysfunction rates after radical prostatectomy have been reported to be 8.9% – 18.3% and 72% – 81%, respectively (Prabhu et al. 2013). Surgical techniques have developed, first after laparoscopic innovations, and later with robotic-assisted laparoscopic prostatectomy (RALP). RALP was associated with improved recovery of erectile function (RR 1.51, 95% CI 1.19-1.92) and continence (RR 1.14, 95% CI 1.04-1.24) compared to laparoscopic operation (Allan and Ilic 2016).

2.4.3 Radiation therapy

Indications for radiation therapy are rather identical with those for radical prostatectomy: curative care due to localized or locally advanced cancer (T1-4, N0-1, M0). There are multiple RCTs comparing radiation therapy dozes and dose increase improved biochemical progression-free survival but there was no impact on overall survival (Dearnaley et al. 2014; Heemsbergen et al. 2014). A propensity-matched retrospective analysis showed dose escalation having an overall survival benefit for men with intermediate or high-risk prostate cancer. Patients with low-

risk prostate cancer did not have an advantage of increased radiation dose (Kalbasi et al. 2015). A Finnish guideline recommends 3D conformal radiation therapy with total dose of 72- 74 Gray (Duodecim 2014).

There is a strong evidence suggesting treating high-risk patients with adjuvant androgen-deprivation therapy (ADT) alongside with radiation therapy. The European Organization for Research and Treatment of Cancer (EORTC) trial randomized patients to receive radiation therapy alone or with 3-year ADT. 10-year clinical disease-free survival was 22.7% in the radiation therapy alone group and 47.7% in the combined treatment group ($p<0.001$). Similar risk decrease was seen for 10-year overall survival (39.8% vs 58.1%, $p=0.0004$) without major adverse effects (Bolla et al. 2010). The radiation therapy combined with ADT is the primary treatment option for locally advanced prostate cancer according to EAU guidelines (Mottet et al. 2017).

Most common adverse effects include bowel and urinary symptoms. Acute rectum irritation causes diarrhea, hemorrhage and rectal discharge. Radiation cystitis results in overactive bladder, dysuria, nocturia and hemorrhage. Acute adverse effects often relieve within 2 – 3 months after radiation therapy. Long-term adverse effects occur usually within 3 years and involve rather similar gastrointestinal and genitourinary symptoms as acute ones. Prevalence of acute and late symptoms was 49% and 21%, respectively, in a retrospective data comparison. Severe late side-effects were rare (prevalence of genitourinary symptoms 4% and gastrointestinal symptoms 2%) (Mohammed et al. 2012).

Brachytherapy is a form of radiation therapy in which a radioactive source is placed into a prostate. There are two types of brachytherapy; low-dose rate and high-dose rate. Low-dose rate therapy involves the insertion of permanent radioactive seeds into the prostate whereas temporary needles are inserted into the prostate for a short period of time at high-dose rate therapy. There are no randomized trials comparing low-dose rate therapy to other curative treatment options but population-based studies suggest that low-dose rate therapy is safe and effective treatment option for localized low-risk or intermediate-risk prostate cancer (Grimm et al. 2012; Sylvester et al. 2011; Taira et al. 2010).

High-dose rate brachytherapy is newer treatment option than low-dose rate therapy and therefore knowledge of its safety and effectiveness is still limited. A randomized trial comparing radiation therapy to radiation therapy combined with high-dose rate brachytherapy showed that the combination had statistically significantly improved the clinical relapse-free survival (10-year estimate of biochemical control of 46% vs 39%, $p=0.04$) but differences in overall survival were

not statistically significant (Hoskin et al. 2012). There are no trials considering high-dose rate brachytherapy as monotherapy but registry studies have observed that high-dose rate therapy seem to be a safe and effective monotherapy treatment option for men with low- and intermediate-risk prostate cancer (Hauswald et al. 2016; Zamboglou et al. 2013).

2.4.4 Androgen-deprivation therapy

Dihydrotestosterone is the main androgen in the prostate and an important factor for prostate cancer progression. Therefore, inhibiting the expression of dihydrotestosterone and other androgens is an efficient method of reducing the progress of prostate cancer and it is the primary treatment for metastatic prostate cancer and additionally it is used as neoadjuvant treatment for radiation therapy.

There are two possibilities to achieve androgen suppression: elimination of testicular androgen secretion (castration) or androgen receptor blockade (antiandrogens). Castration can be achieved by bilateral subcapsular orchiectomy. Other option is to use gonadotropin-releasing hormone (GnRH) agonist or antagonist (chemical castration). The castrate testosterone level has been defined to be less than 1.73 nmol/l. However, there were inferior 5-year biochemical recurrence-free survival in men with testosterone level at 1.1-1.7 nmol/l compared to men with the level less than 1.1 nmol/l (Pickles et al. 2012).

Surgical and chemical castrations are equally efficient and safe (Seidenfeld et al. 2000). Often surgical treatment is offered to elder men since it is a quicker method to achieve castration level than the chemical one (Loblaw et al. 2004). GnRH-agonists are usually delivered as depot-injections and it is recommended to treat the patient with antiandrogens after the first GnRH-agonist injection due to flare phenomenon (transient increase in testosterone level), especially if there are widespread bone metastases (Kuhn et al. 1989). GnRH-antagonist provide quick castration level without flare-up reaction but the disadvantage is monthly injection rate. Side-effects are similar among all castration options: hot waves, sweating, muscle atrophy, osteoporosis, sexual dysfunction, anemia and increased risk of metabolic syndrome.

Antiandrogen treatment is a considerable alternative to castration, especially for sexually active younger patients without widespread bone metastases. Monotherapy with bicalutamide has been shown to be as effective as castration in men with non-metastatic locally advanced prostate cancer. In addition, there were statistically

significant decrease in side-effects (sexual interest and physical capacity) (Iversen et al. 2000). Classic adverse effect of bicalutamide is breast tenderness due to testosterones increased aromatization to estrogen. Therefore, prophylactic irradiation of breasts is provided before initiation of antiandrogen monotherapy. In Finland, another non-steroidal antiandrogen in clinical use is flutamide. Combining castration with antiandrogen treatment provides slight improve in 5-year survival (HR 0.87, 95% CI 0.81-0.94) but there were more withdrawals from treatment among men receiving combined medication (Samson et al. 2002).

Intermittent alternative has been developed to reduce the adverse effects of continuous ADT. A meta-analysis including 15 trials and 6856 patients reported that there was no statistically significant difference between continuous and intermittent ADT for overall survival (HR 1.02, 95% CI 0.93-1.11) or prostate cancer-specific survival (HR 1.02, 95% CI 0.87-1.19). However, men in intermittent therapy had less adverse effects, for example hot flashes (RR 0.76, 95% CI 0.57-1.00) and cardiovascular death (RR 0.86, 95% CI 0.73-1.02) compared to men in continuous therapy (Magnan et al. 2015).

2.4.5 Treatment of castration resistant prostate cancer

2.4.5.1 Chemotherapy

Chemotherapy is indicated treatment if there is an active metastatic disease despite castration. Docetaxel-based chemotherapy given every three weeks was associated with improved survival ($p=0.009$) compared to men receiving mitoxantrone. Median survivals were 18.9 and 16.5 months, respectively (Tannock et al. 2004). Men with long progression-free period after first-line docetaxel benefit from subsequent treatment with docetaxel (Loriot et al. 2010). The most common adverse effects include neutropenia, nausea, hand-foot syndrome and mucosal atrophy.

Cabazitaxel has advantageous effect on docetaxel-resistant cancers. Median survivals were 15.1 and 12.7 months ($p < 0.0001$) among men with cabazitaxel and mitoxantrone, respectively (de Bono et al. 2010). Adverse effects are similar with docetaxel.

2.4.5.2 Androgen targeted therapy

Second generation antiandrogen enzalutamide has been shown to be efficacious in treatment for castrate resistant prostate cancer both as first-line treatment and after docetaxel (Beer et al. 2014; Scher et al. 2012). Similar findings have been made for another androgen biosynthesis inhibitor abiraterone. The intervention group without previous docetaxel treatment had median overall survival of 34.7 months whereas the placebo group had median survival of 30.3 months ($p = 0.0033$) (Ryan et al. 2015). In addition, abiraterone was beneficial after docetaxel treatment if there was cancer progression. Median overall survival was 15.8 months for the abiraterone group and 11.2 for the placebo group ($p < 0.0001$) (Fizazi et al. 2012). The most usual adverse effects include fatigue, hypertension, dizziness and lower back pain for enzalutamide and cardiac disorders, hypokalemia and elevated liver enzyme levels.

2.4.5.3 Alpha-emitted therapy

Radium-223 (Alfarad) has been shown to be effective for patients with bone metastasis. It binds to tissues with increased bone metabolism and hence has high affinity for bone metastasis but not for visceral metastasis. Men receiving six radium-223 injections had statistically significant improvement in overall survival (HR 0.70, 95% CI 0.58-0.83) compared to the placebo group (Parker et al. 2013). However, high price restricts radium-223 use. Another serious adverse effect is myelotoxicity.

2.4.5.4 Bone targeted agents

Zoledronic acid (bisphosphonate) has been used to prevent bone fractures. The intervention group (4 mg zoledronic acid every 3 weeks) had less skeletal-related events than the placebo group (33.2% vs 44.2%, $p=0.021$) in a randomized study but there was no differences in quality-of-life scores or disease progression (Saad et al. 2002).

However, a modern receptor activator of nuclear factor-kB ligand (RANKL) inhibitor denosumab has been showed to be superior when compared to zoledronic acid. Median time to first skeletal-related effect was 20.7 months among denosumab group and 17.1 months among zoledronic acid group ($p = 0.008$) (Fizazi et al. 2011). The most serious but rare adverse effect is osteonecrosis of the jaw.

2.5 Prostate cancer and pharmacoepidemiology

A summary of medications with a possible association with prostate cancer is presented in Table 6. Associations between use of digoxin and beta-blockers and cancer are discussed in greater detail in chapters 2.6.3 and 2.7.2.1.

2.5.1 5-alpha-reductase inhibitors (5-ARIs)

5-ARIs inhibit testosterone conversion to dihydrotestosterone, which is the primary androgen in the prostate. The primary indication for 5-ARI use is the treatment of BPH and the two 5-ARIs sold in Finland are finasteride and dutasteride (Kela and Fimea 2017). The Prostate Cancer Prevention Trial (PCPT) randomized 18,882 previously healthy men, aged 55 years or older, to receive finasteride or placebo. After 7 years of follow-up, cumulative incidence of prostate cancer was 18.4% in the finasteride group and 24.4% in the control group ($p < 0.001$). However, there was a higher percentage of high-grade tumors in the finasteride arm. Cumulative incidence was relatively high mainly due to biopsy protocol of the trial; PSA concentration was measured annually, and because use of finasteride lowers PSA level, the PSA values among finasteride arm were first doubled. However, at the start of the participants' fourth year in the study, the factor was changed to 2.3 aiming to an equal percentage of biopsies in both groups. Men with a PSA value higher than 4.0 ug/l were referred to prostate biopsy (I. M. Thompson et al. 2003). A randomized trial comparing dutasteride to placebo showed similar results. In the RCT of 8,231 men aged between 50-75 years, dutasteride use was associated with decreased prostate cancer risk (RR 0.77, 95% CI 0.70-0.85), but there were more high-grade cancers over the 4-year study period (Andriole et al. 2010). 5-ARI use was not associated with prostate cancer survival (HR 0.94, 95% CI 0.72-1.24 and HR 0.98, 95% CI 0.69-1.41 for usage before and after the diagnosis, respectively) among men with prostate cancer in the FinRSPC (Murtola et al. 2016).

2.5.2 Statins

Statins are used to lower low-density lipoprotein (LDL) concentration and thus to reduce the risk of cardiovascular diseases. Statins sold in Finland are simvastatin, atorvastatin, fluvastatin, rosuvastatin, lovastatin and pravastatin. Use of statins is common and for example 42% of study population in the FinRSPC had used statins

during the 16 year study period (Kaapu et al. 2016). Statin use was associated with slightly reduced prostate cancer risk in a Danish case-control study of 254,880 men; adjusted OR 0.94, 95% CI 0.91-0.97 for statin use compared with non-use. Risk reduction was larger for risk of advanced prostate cancer, adjusted OR 0.90, 95% CI 0.85-0.96 and with statin use of 10 years or more, adjusted OR 0.78, 95% CI 0.65-0.95 (Jespersen et al. 2014). Similar results were obtained in the FinRSPC cohort. Overall prostate cancer risk was lower among statin users (HR 0.75, 95% CI 0.63-0.89) when compared with non-users of statins. The association was in the same direction between long-term use (6 years or more) and prostate cancer risk but not statistically significant (HR 0.70, 95% CI 0.45-1.08) (Murtola et al. 2010). Consistent but statistically non-significant results were observed in an American cohort study of 32,091 men aged between 40-79 at baseline. Compared to non-use, there was no statistically significant association between statin use and overall risk of prostate cancer (HR 0.86, 95% CI 0.63-1.18) The association was stronger, but nevertheless non-significant, for high-grade cancer (HR 0.62, 95% CI 0.30-1.28) (Kantor et al. 2015). The REDUCE study differed from the studies mentioned above; statin use did not modify prostate cancer risk (Freedland et al. 2013). There are no published reviews concerning statin use and risk of prostate cancer.

A meta-analysis of 34 cohort studies showed statin use was associated with a reduction in both risk of all-cause mortality and prostate cancer-specific mortality (HR 0.76, 95% CI 0.63-0.91 and HR 0.76, 95% CI 0.64-0.89, respectively). Statin use was associated with decreased risk of biochemical recurrence among men treated with radiation therapy (HR 0.79, 95% CI 0.65-0.95) but not with radical prostatectomy (HR 0.94, 95% CI 0.81-1.09) (Raval et al. 2016). Randomized trials are required to achieve further information about statins and prostate cancer.

2.5.3 Metformin and other anti-diabetic drugs

Metformin is the first-line medication for the treatment of type 2 diabetes mellitus. The association between metformin use and prostate cancer is widely studied, and the results are inconsistent. Compared to non-users, metformin users had a reduced prostate cancer risk in a Danish case-control study (Preston et al. 2014) and in a Finnish cohort study (Haring et al. 2017), whereas there was no association between use of metformin and prostate cancer risk in the REDUCE study (T. Feng et al. 2015) and a Canadian cohort study (Margel et al. 2013). A meta-analysis of 18 cohort or case-control studies with 52,328 cases concluded that metformin use was not

statistically significantly associated with prostate cancer risk (RR 0.97, 95% CI 0.80-1.16) (Z. Feng et al. 2019). Similar conclusion was made in another meta-analysis, which included 18 cohort studies and 6 case-control studies with 2,009,504 men. There was no association between prostate cancer risk and use of metformin (HR 0.97, 95% CI 0.84-1.12 in case-control studies and HR 0.94, 95% CI 0.79-1.12 in cohort) (Y. Wang et al. 2019).

Metformin users had borderline significant reduction in the biochemical recurrence risk (HR 0.82, 95% CI 0.76-1.01) in a systematic review. Metformin use was associated neither with prostate cancer-specific mortality (HR 0.76, 95% CI 0.46-1.33) nor all-cause mortality (HR 0.86, 95% CI 0.67-1.10) (Raval et al. 2015). In a separate review, prostate cancer patients with diabetes not using metformin were at higher prostate cancer recurrence risk (HR 1.20, 95% CI 1.00-1.44) (Hwang et al. 2015).

A meta-analysis of 10 cohort studies and one case–control study evaluated prostate cancer risk among men receiving insulin therapy. A total number of 205,523 men with 7,053 prostate cancer cases were included. Prostate cancer risk among insulin users did not differ from men using other glucose-lowering agents (RR 0.89, 95% CI 0.72-1.09) (Y. Chen et al. 2013).

2.5.4 Other suggested agents besides antiarrhythmic drugs

Aspirin and nonsteroidal anti-inflammatory drugs (NSAIDs) have been suggested to be associated with a reduced prostate cancer risk. Several population-based studies have found an association between aspirin/NSAID use and decreased prostate cancer incidence (Doat et al. 2017; Platz et al. 2005a; Vidal et al. 2015). However, conflicting results have been published and further studies are required (Veitonmaki et al. 2014). NSAID users had a decreased risk of prostate cancer (RR 0.89, 95% CI 0.81-0.98) in a meta-analysis of 43 studies. In addition, use of aspirin was associated with a decreased prostate cancer risk (RR 0.93, 95% CI 0.89-0.96) (Shang et al. 2018).

Antiepileptic drugs, especially valproate, have been suggested to have antineoplastic properties due to histone deacetylase inhibitory features (Batta et al. 2007). Salminen et al. found diminished prostate cancer risk among valproate, phenobarbital and carbamazepine users (Salminen et al. 2016). Nonetheless, contradictory results have been observed and again, additional studies are necessary (Kang et al. 2014).

In addition, association between antihypertensive drugs and prostate cancer risk has been researched. A meta-analysis of 12 cohort and 9 case-control studies showed that use of angiotensin converting enzyme (ACE) inhibitors, angiotensin receptor blocker (ATRB) or diuretics had no influence on prostate cancer risk. Calcium channel blocker (CCB) use increased prostate cancer incidence (RR 1.08, 95% CI 1.00-1.16) (Cao et al. 2018a).

Table 6. Summary of medications possibly associated with prostate cancer (digoxin and beta blockers excluded).

Name of drug	Preclinical study	Epidemiological study	Clinical Study	Summary
5-ARIs	Decreased DHT-concertation might be beneficial for prostate[1].	Use of 5-ARIs did not affect prostate-cancer specific survival[2].	Users of 5-ARIs had a decreased prostate cancer risk but more high-grade cancers occurred[3,4].	5-ARIs are not recommended for prostate cancer prevention.
Statins	Statins might inhibit growth of prostate cancer via several pathways[5].	Users of statins seem to have a reduced prostate cancer risk, especially for high-grade cancer[6,7].	Long-term use of atorvastatin might lower prostate cancer proliferation rate[8].	Further evidence from RCTs is required.
Metformin	Metformin was observed to inhibit growth of prostate cancer cells[9].	Metformin use did not have impact on prostate cancer risk in the meta-analysis of studies[10].	-	Potential anti-neoplastic properties have not been observed consistently in population-based studies.

Insulin	The presence of insulin receptors on prostate cancers has been observed[11].	Insulin use was not associated with prostate cancer risk in a meta-analysis[12].	-	Use of insulin does not have an effect on prostate cancer risk.
NSAIDs	Cyclooxygenase-2 has been observed to be overexpressed in prostate carcinoma[13].	NSAID use decreased risk of prostate cancer in a meta-analysis of 43 studies[14].	-	Further evidence from RCTs is required.
ACE-inhibitors	ACE-inhibitors might inhibit angiogenesis in prostate cancer cells[15].	ACE inhibitor use and the risk of prostate cancer were not associated according to a meta-analysis of 10 studies[16].	-	It is likely that ACE-inhibitors do not have impact on prostate cancer.
ATRBs	ATRBs block AT receptors, which might suppress activity of cell proliferation in prostate cancer[17].	There was no significant association between ATRB usage and the risk of prostate cancer in a meta-analysis of 5 studies[16].	-	ATRBs probably do not have an effect on prostate cancer.
CCBs	CCB increased intracellular Ca^{2+}-concentration, which	CCB users had a slightly increased risk of prostate cancer according to a	-	CCB might increase prostate cancer risk but

	might lead to apoptosis, in prostate cancer cells[18].	meta-analysis of 14 studies[16].		the risk increase is minor.
Valproate	Valproate treatment resulted in decreased net proliferation rate of prostate cancer cells[19].	Contradictory results have been published[20,21].	Valproate might decrease PSA among men with castration resistant prostate cancer[22].	Valproate might be beneficial but further studies are required.

DHT = Dihydrotestosterone, 5-ARI = 5-alpha-reductase inhibitors, RCT = Randomized controlled trial, NSAID = Non-steroidal anti-inflammatory drugs, ACE = Angiotensin converting enzyme, ATRB = Angiotensin receptor blocker, CCB = Calsium channel blocker, PSA = Prostate-specific antigen

1 (Lonergan and Tindall 2011).
2 (Murtola et al. 2016).
3 (I. M. Thompson et al. 2003).
4 (Andriole et al. 2010).
5 (Shibata et al. 2003).
6 (Jespersen et al. 2014).
7 (Murtola et al. 2010).
8 (Murtola et al. 2018).
9 (Zakikhani et al. 2008).
10 (Y. Wang et al. 2019).
11 (Cox et al. 2009).
12 (Y. B. Chen et al. 2013).
13 (Gupta et al. 2000).
14 (Shang et al. 2018)
15 (Egami et al. 2003).
16 (Cao et al. 2018a).

17 (Uemura et al. 2003).
18 (Jan et al. 2001).
19 (Xia et al. 2006).
20 (Salminen et al. 2016).
21 (Kang et al. 2014).
22 (Sharma et al. 2008).

2.6 Digoxin

2.6.1 Mechanism of action and traditional indications

Cardiac glycosides, of which digoxin is the most commonly used, are inotropic agents. They increase stroke volume, and thus enlarges cardiac output. Additionally, cardiac glycosides decrease heart rate. Above-mentioned properties make digoxin and other cardiac glycosides, such as digitoxin, optimal drug for congestive heart failure, especially, if patient has an atrial fibrillation or flutter, and therefore, indications for digoxin use are decreasing heart rate in atrial fibrillation or improving cardiac output in chronic heart failure (Ruskoaho et al. 2014).

Digoxin binds to alpha unit of an enzyme called sodium-potassium adenosine triphosphatase (Na^+/K^+ -ATPase). The Na^+/K^+ -ATPase transports sodium out of the cell and exchanges it to potassium. Digoxin decreases activity of the Na^+/K^+ -ATPase resulting in increased intracellular sodium concentration. This inhibits the sodium-calcium exchanger, which substitutes intracellular calcium with extracellular sodium. The cascade leads to increased intracellular calcium concentration, which intensifies the force of contraction of the heart. Furthermore, increased calcium concentration slows the heart rate via lengthening the cardiac action potential (Ruskoaho et al. 2014).

Digoxin has systemic effects also. It increases sensitivity to vagal stimulation and activates vagal centers in the central nervous system, and therefore intensifies parasympathetic nervous system activation (Ruskoaho et al. 2014).

2.6.2 Potential antineoplastic mechanisms of digoxin

2.6.2.1 Na^+/K^+ -ATPase

Inhibition of Na^+/K^+ -ATPase increases intracellular Ca^{2+}-concentration as discussed above. Calcium has a vital role in activating programmed cell death (apoptosis) (McConkey and Orrenius 1997). Na^+/K^+ -ATPase is also expressed at tumor cells (Repke 1988). A previous study has shown that injecting thapsigargin (inhibitor of Ca^{2+}-APTase) to prostate cancer cell increases Ca^{2+}-concentration,

likewise digoxin, and activates apoptosis (Furuya et al. 1994). An *in vitro* study observed digoxin injections inducing apoptosis in androgen-independent human prostate cancer cell lines and concluded that increased intracellular Ca^{2+}-concentration was the most important trigger for apoptosis (McConkey et al. 2000). Furthermore, digoxin decreases intracellular K^+-concentration via inhibiting Na^+/K^+ -ATPase and decreased K^+-concentration is another apoptosis trigger (Hughes et al. 1997).

2.6.2.2 HIF-1alpha

Hypoxia-inducible factor 1-alpha (HIF-1alpha) is a subunit of transcription factor HIF-1, which is an essential gene activator for tumor invasion, angiogenesis and cell survival (Harris 2002). Intratumoral hypoxia and oncogene mutations induce overexpression of HIF-1alpha in human cancers, which has also been identified as a risk factor for treatment failure (Semenza 2003). Digoxin and other cardiac glycosides do not inhibit the expression of HIF-1alpha mRNA, but the translation of mRNA into protein (Zhang et al. 2008). Reduced HIF-1alpha expression and xenograft tumor growth were observed both *in vitro* and *in vivo* after digoxin injections (Zhang et al. 2008).

2.6.2.3 Estrogenic effects

There are two estrogen receptors (ER) in the human prostate, ER-alpha and -beta. Activation of the beta receptor restricts cell proliferation in prostate cancer and therefore it might be possible to inhibit disease progression to the castration-resistant form via ER-beta (Bonkhoff 2018; Carruba 2007; Thelen, Wuttke, Seidlová-Wuttke 2014). Since the chemical structure of digoxin is rather similar to estradiol, it has affinity for estrogen receptors too (Rifka, Pita, Loriaux 1976; Rifka et al. 1978). Phyto-estrogenic effect might have impact on risk of estrogen-sensitive cancers, for example breast cancer. A review considering 18 studies found that women consuming high doses of phytoestrogens might have a reduced breast cancer risk (Peeters et al. 2003). A meta-analysis containing 23 studies and a total of 11,346 cases and 140,177 controls showed that there was an association between use of daidzein (OR 0.85, 95% CI 0.75-0.96), genistein (OR 0.87, 95% CI 0.78-0.98) and glycitein (OR 0.89, 95% CI 0.81-0.98) and decreased prostate cancer risk. However, use of

total isoflavones (OR 0.93, 95% CI 0.84-1.04) and several other separate isoflavones lacked the association (Q. Zhang et al. 2017).

2.6.3 Digoxin use and cancer

2.6.3.1 Digoxin use and overall cancer risk

Even though there are only few studies on digoxin use and overall cancer risk, there are numerous studies on individual cancer types and digoxin. A Norwegian case-control study reported an increased overall cancer incidence among digitoxin users among study population consisting of both men and women (OR 1.21, 95% CI 1.15-1.28) (Haux et al. 2001a).

The most widely studied cancer is breast cancer; two rather identical meta-analyses have been published on the breast cancer risk and cardiac glycoside use. Both included 9 studies, of these 8 were the same in both reviews. Results are very similar: cardiac glycoside use was associated with an increased risk of breast cancer (RR 1.33, 95% CI 1.25-1.42 and RR 1.34, 95% CI 1.25-1.44) with no significant heterogeneity between studies. Digoxin users have especially high risk for ER-positive breast cancer compared to non-users (Karasneh, Murray, Cardwell 2017; Osman et al. 2017).

A meta-analysis on the risk of colorectal cancer and cardiac glycoside use included four studies but only one of them considered digoxin, while the others studied digitalis or digitoxin. The conclusion was that cardiac glycoside users had an increased risk of colorectal cancer (RR 1.38, 95% CI 1.20-1.58) (Osman et al. 2017). A large Swedish cohort study, published after the meta-analysis, had coherent results. After adjustment for age, sex, residence and comorbidities, digoxin users had an increased colorectal cancer incidence (HR 1.24, 95% CI 1.18-1.30). However, the risk increase was associated with short-time use of digoxin and disappeared in long-term use (Xie et al. 2017).

Digoxin use was associated with an increased lung cancer risk in a meta-analysis of three studies (RR 1.32, 95% CI 1.03-1.69) (Osman et al. 2017). Similar findings were obtained in a Taiwanese study: digoxin users had an increased lung cancer risk (HR 1.38, 95% CI 1.11-1.70) after an 8-year follow-up. Nonetheless, after adjustment for age, sex, region, income, urbanization and Charlson comorbidity index (CCI), the association was not statistically significant (HR 1.20, 95% CI 0.98-1.51) (Chung et al. 2017).

There are several cohort and case-control studies reporting of digoxin use and a risk of separate cancer types. The studies are listed in Table 7.

2.6.3.2 Digoxin use and overall cancer prognosis

There are no published prospective studies on digoxin use and overall cancer survival. However, some publications have addressed digoxin use and cancer survival in specific cancer types and even a meta-analysis has been published based on these studies (Osman et al. 2017). The meta-analysis contained six cohort studies: two on prostate cancer and one on colorectal cancer, breast cancer, epithelial ovarian cancer and glioblastoma each. All reported all-cause mortality and four of them also reported cancer-specific mortality. Digoxin users had a decreased overall survival (HR for death 1.35, 95% CI 1.25-1.46) but there was no association between digoxin use and a cancer-specific survival (HR 1.08, 95% CI 0.97-1.19) (Osman et al. 2017). The studies are listed in Table 8.

Table 7. Epidemiological studies on digoxin and cancer risk (prostate cancer, breast cancer, colorectal cancer and lung cancer excluded).

Cancer type	Study	Study design	Size of study sample	RR (95% CI)
Glioblastoma				
	Boursi et al	Case-control	5329	0.80 (0.40-1.59)
	Seliger et al	Case-control	22,055	0.74 (0.36-1.55)
Uterine				
	Biggar et al	Cohort	2,116,029	1.48 (1.32-1.62)
Ovarian				
	Biggar et al	Cohort	2,116,029	1.06 (0.92-1.22)
Cervical				
	Biggar et al	Cohort	2,116,029	1.00 (0.79-1.25)
Esophagus				
	Xie et al	Cohort	708,285	1.22 (1.01-1.46)
Stomach, cardia				
	Xie et al	Cohort	708,285	1.04 (0.80-1.36)
Stomach, non-cardia				
	Xie et al	Cohort	708,285	0.90 (0.78-1.05)
Small intestine				
	Xie et al	Cohort	708,285	1.00 (0.76-1.33)
	Xie et al	Cohort	708,285	1.06 (0.88-1.29)
Gallbladder				
	Xie et al	Cohort	708,285	1.63 (1.19-2.25)
Pancreas				
	Xie et al	Cohort	708,285	1.08 (0.94-1.24)

Table 8. Epidemiological studies on digoxin and cancer prognosis.

Cancer type	Study	Size of study sample	HR (95% CI), overall survival	HR (95% CI), cancer-specific survival
Prostate cancer				
	Flahavan et al	5,732	1.24 (1.07-1.43)	1.13 (0.91-1.42)
	Karasneh et al	13,134	1.39 (1.23-1.56)	1.13 (0.93-1.37)
Colorectal cancer				
	Karasneh et al	10,357	1.53 (1.34-1.73)	1.10 (0.91-1.34)
Glioblastoma				
	Boursi et al	1,076	1.56 (0.80-3.04)	-
Epithelial ovarian cancer				
	Vogel et al	762	1.29 (0.81-2.06)	-
Breast cancer				
	Karasneh et al	17,842	1.24 (1.08-1.41)	0.91 (0.72-1.14)

2.6.3.3 Digoxin use and the risk of prostate cancer

Several epidemiological studies on cardiac glycosides and prostate cancer risk have been published. A study, including 9,271 digitoxin users and one control for each drug user, reported increased prostate cancer risk among the drug users compared to non-users (OR 1.25, 95% CI 1.08-1.45) (Haux et al. 2001b). In a large prospective cohort study of 47,884 men, there was a significant association between current digoxin use during the follow-up and a reduced prostate cancer risk (RR 0.78, 95% CI 0.67-0.90). The association was strongest for long-term (over 10 years) use (RR

0.54, 95% CI 0.37-0.79) (Platz et al. 2011). An U.S. case-control study had slightly incoherent results; digoxin use was not statistically significantly associated with the prostate cancer incidence (OR 0.58, 95% CI 0.30-1.10) and long-term users had a higher prostate cancer risk than short-term users. However, prostate cancer risk was decreased among digoxin users with 3 or more PSA-tests during the past five years (OR 0.44, 95% CI 0.20-0.98) (Wright, Hansten, Stanford 2014). There was no association between digoxin use and the prostate cancer risk (HR 0.89, 95% CI 0.67-1.18) in a Taiwanese cohort study (L. T. Kao et al. 2018).

A meta-analysis containing the studies mentioned above along with the two studies included in this doctoral thesis observed no association between digoxin use and the prostate cancer risk (RR 1.02, 95% CI 0.87-1.19), but there was a significant heterogeneity in the results; in contrast to others, the case-control study published by Haux et al 2001 observed increased prostate cancer risk among digoxin users. This is probably due to lack of adjustments for confounders. There was some suggestion that long-term use (5 years or longer) of digoxin might lower prostate cancer risk (RR 0.89, 95% CI 0.79-1.02) and the risk of cancer with Gleason score 7 or more was diminished (RR 0.80, 95% CI 0.68-0.96) (Osman et al. 2017).

2.6.3.4 Digoxin use and prostate cancer prognosis

Two studies considering digoxin use and prostate cancer prognosis have been published. The first followed 5,732 men with prostate cancer for a median follow-up of 4.3 years. Men with digoxin exposure prior to prostate cancer diagnosis had a statistically significant increase in all-cause mortality, but not in prostate cancer-specific mortality (HR 1.24, 95% CI 1.07-1.43 and HR 1.13, 95% CI 0.91-1.42, respectively) (Flahavan et al. 2014).

The second study identified 13,134 prostate cancer cases with a mean follow-up of 5.0 years. Digoxin use after prostate cancer diagnosis was not associated with prostate cancer-specific survival (HR 1.13, 95% CI 0.93-1.37) but all-cause mortality was increased among digoxin users compared to non-users (HR 1.39, 95% CI 1.23-1.56), mainly due to cardiovascular deaths (HR 1.85, 95% CI 1.49-2.31) (Karasneh et al. 2016).

2.7 Other antiarrhythmic drugs

2.7.1 Antiarrhythmic drugs classification

Antiarrhythmic agents are divided into four separate groups by mechanism of action.

Class I includes Na^+-channel blockers and there are three subclasses Ia (lengthens repolarization), Ib (shortens repolarization) and Ic (slows electrical conduction). Agents belonging to class Ia are quinidine and disopyramide, to class Ib lidocaine and mexiletine and to class Ic flecainide and propafenone.

Class II drugs are beta-blockers (BBs). We did not include pure BBs in the cohort studies, as they are studied together with antihypertensive agents (Siltari et al. 2018).

Agents blocking K^+-channel form class III. The agents prolong duration of action potential, repolarization and refractory period. Class III includes amiodarone, dronedarone, sotalol, ibutilide and dofetilide.

Slow Ca^{2+}-channel blockers belong to class IV. The only drugs in clinical use from this group are verapamil and diltiazem. Ca^{2+}-channel blockers are commonly used as antihypertensive agents and we did not include them into the study.

In addition, there are drugs with antiarrhythmic properties not fitting any traditional antiarrhythmic agent class. Therefore, class V has been developed. It contains digoxin (discussed previously), adenosine (activates K^+-channels in the atrium and in the sinus node and thus shortens the duration of action potential) and novel agents such as vernakalant and ivabradine.

2.7.2 Antiarrhythmic drugs and cancer

2.7.2.1 Beta-blockers and cancer

Beta-adrenergic activation is vital to several hallmarks of cancer and therefore, BBs have been hypothesized to have potential to prevent cancer progression (Cole and Sood 2012). Many observational studies have been published on risk of cancer death among BB users, summarized in meta-analyses.

A review covering 24 epidemiological studies found that pre-diagnostic BB use had no influence on either all-cause mortality or cancer-specific mortality (HR 1.04, 95% CI 0.95-1.13 and HR 0.91, 95% CI 0.84-1.00, respectively). In addition, pre-diagnostic BB use was not statistically significantly associated with prostate cancer-

specific death (HR 0.87, 95% CI 0.71-1.07). However, compared to non-users, post-diagnostic BB users had a reduced all-cause mortality (HR 0.89, 95% CI 0.81-0.98) and cancer-specific mortality (HR 0.89, 95% CI 0.79-0.99). The association was not statistically significant for prostate cancer-specific death only (HR 0.84, 95% CI 0.70-1.01) (Zhong et al. 2016).

Choi et al. 2014 included 12 epidemiological studies and BB use was associated with improved overall survival (HR 0.79, 95% CI 0.67-0.93) and prolonged disease-free survival (HR 0.69, 95% CI 0.53-0.91). There was no association between beta blocker use and prostate cancer death (HR 0.61, 95% CI 0.23-1.60) (Choi et al. 2014).

Interestingly, immortal time bias, which is discussed more in chapter 6, might explain the observed beneficial risk associations in observational studies. A meta-analysis of 30 studies showed a reduced risk of death among BB users compared to non-users (HR 0.88, 95% CI 0.79-0.97). Nevertheless, after excluding 11 studies potentially prone to immortal time bias, there was no association between BB use and overall survival (HR 1.00, 95% CI 0.93-1.07). A similar phenomenon was seen with cancer-specific survival (Weberpals et al. 2016).

A review, including 8 epidemiological studies (2 cohort studies and 6 case-control studies), summarized possible association between BB use and prostate cancer risk. According to the review, BB use had no statistically significant association with the risk of prostate cancer (HR 0.91, 95% CI 0.81-1.02) but there was significant heterogeneity between the studies; one of the case-control studies reported significantly increased prostate cancer risk, which might be due to systematic difference between BB users and non-users, more frequent PSA measurement interval or immortal time bias, for example (Cao et al. 2018b).

2.7.2.2 Other antiarrhythmic drugs and cancer

Studies about cancer and antiarrhythmic agents besides digoxin and BBs are sparse. Amiodarone might increase cancer risk. A Taiwanese cohort study found an increased overall cancer risk among high-dose (Defined Daily Dose (DDD) > 180) amiodarone users compared with the general population (SIR 1.28, 95% CI 1.00-1.61). However, there was no clear association between overall amiodarone use and cancer risk (SIR 1.12, 95% CI 0.99-1.26) (Su et al. 2013).

Another Taiwanese study, a case-control study of 9,944 cases and 19,497 matched controls, observed an increased liver and intrahepatic bile duct cancer incidence among amiodarone users compared to non-users (OR 1.60, 95% CI 1.45-1.77). Compared to non-uses, the risk increase was even larger among patients with high-

dose (DDD > 145) amiodarone use (OR 1.79, 95% CI 1.50-2.15). Other antiarrhythmic agents (mexiletine, propafenone, quinidine and procainamide) had no impact on the risk of liver and intrahepatic bile duct cancer (Lim et al. 2015).

A third Taiwanese study investigated antiarrhythmic drug use and prostate cancer risk. None of the observed drug groups associated with prostate cancer risk; HRs for Na^+-channel blockers, K^+-channel blocker and Ca^{2+}-channel blockers were 1.12 (95% CI 0.84-1.50), 0.89 (95% CI 0.59-1.34) and 1.14 (95% CI 0.95-1.36), respectively (Kao et al. 2017).

A U.S. cohort study of 93,265 patients assessed Na^+-channel inhibitors and cancer survival. There was some suggestion that users of Class I antiarrhythmic drugs had more favorable overall survival compared to non-users (HR 1.11, 95% CI 0.98-1.24) (Fairhurst et al. 2015).

3 AIMS OF THE STUDY

Previous epidemiological studies considering digoxin and prostate cancer have provided slightly inconsistent results with diverse study population and methods. We wanted to study the possible association between digoxin and other antiarrhythmic drug use and cancer among large population-based study population with robust information on medication use. The objective of our study is to explore whether use of digoxin or other antiarrhythmic drugs associates with prostate cancer risk, prostate cancer survival or overall cancer mortality at population level. The specific aims are:

1. To evaluate the association between antiarrhythmic drug use and prostate cancer risk.
    a. Moreover, to evaluate the impact of cumulative dose, length and intensity of antiarrhythmic drug use.
    b. To evaluate prostate cancer risk separately by grade and stage.
    c. To evaluate the role of screening in the risk association.

2. To estimate the association between prostate cancer survival and antiarrhythmic drug use before and after prostate cancer diagnosis in a cohort study.
    a. Additionally, to evaluate survival trends by amount, duration and intensity of post-diagnostic digoxin and sotalol use.
    b. To evaluate effect modification by age, tumor characteristics, screening trial arm, usage of other drug groups and primary treatment.

3. To evaluate the association between antiarrhythmic drug use and overall cancer mortality in a cohort study.
    a. To further evaluate the risk of death from most frequent cancer types (lung, colorectal, pancreatic, gastric, liver, renal, non-Hodgkin lymphoma, bladder and central nervous system cancer).

b. To evaluate the effect of cumulative amount, duration and intensity of antiarrhythmic drug use.
c. To evaluate effect modification by age, baseline cancer and use of other drug groups.

4 SUBJECTS AND METHODS

4.1 Data sources

4.1.1 Finnish Cancer Registry

The Finnish Cancer Registry, established in 1953, is a population-based institute maintained by the Cancer Society of Finland. All physicians are required by a sub-law to inform the national Cancer Registry about the primary site of cancer, cancer stage, method of diagnosis and primary treatment. In addition, pathological laboratories have a similar obligation; information on tumor histology must be transferred to the Cancer Registry also. Cause-of-death information from the Statistics Finland and the data from The Care Register for Health Care are updated annually to the Cancer Registry. Based on information from different sources, each cancer case is summarized, and the data can be used for research. Information on practically all cancer cases diagnosed in Finland is transferred to the Cancer Registry, the coverage for solid tumors was estimated to be 96%, and for non-solid tumors 86% (Leinonen et al. 2017; Pukkala et al. 2018).

4.1.2 Population Register Center

The Population Register Center maintains the Population Information System, which is a large data repository containing basic information about all Finnish citizens and foreign citizens residing permanently in Finland. The data include information on name, national identification number, address, citizenship, native language, family relations and date of birth and, if occurred, death. In addition to research, the information from the Population Information System is used for taxation, elections, and judicial administration, for example (Population Register Center).

4.1.3 Social Insurance Institution of Finland Prescription Register

The Social Insurance Institution (SII) is a government agency, providing social security for Finnish residents. Benefits provided by SII include reimbursement for the cost of prescribed medicines when purchased from the pharmacy. The Pharmaceuticals Pricing Board, operating under the Ministry of Social Affairs and Health, controls which drugs can be reimbursed. Currently, there are three different reimbursement categories after the annual deductible of 50 euros (the deductible was introduced 1.1.2016. There was a deductible of 10 euros for each purchase until 2005); basic rate of reimbursement is 40%, lower special rate of reimbursement is 65% and higher special rate of reimbursement is 100%. The reimbursement category depends on the indication of medication use and thus, the same agent might have different reimbursement rate depending on the indication. Digoxin and other antiarrhythmic drugs have the lower special rate of reimbursement.

To be justified in special rate of reimbursement, patient must apply for entitlement to higher than regular reimbursement which requires a medical certificate issued by a doctor on form B. In addition, drug producers are not obligated to apply for reimbursement status and therefore all purchases of antiarrhythmic drugs are not included in our study material. For example, digoxin was removed from the reimbursement system at the beginning of 2013 but afterwards it was returned to the system.

The reimbursement system provides an opportunity to maintain a comprehensive prescription database, which includes information on reimbursed drug and its Anatomical Therapeutic Chemical code, date for each reimbursed purchase, number of packages acquired and the number and strength of pills (Klaukka 2009). All Nordic prescription databases are in active research use and provide accurate information for pharmacoepidemiologic studies (Wettermark et al. 2013).

4.1.4 Finnish Randomized Study of Screening for Prostate Cancer

The FinRSPC started over 20 years ago and still provides useful data for researchers. The overall study population was 80,458 (but 312 were excluded because of death, prevalent prostate cancer or emigration) Finnish men, aged 55-67 years at entry. The men were identified from the Population Register Center and each year in 1996-1999 8,000 men were randomized to the screening arm and the remaining roughly 12,000 men formed the control arm. All men in the trial were followed up for cancer incidence through the national Finnish Cancer Registry. Men in the screening arm

were invited to PSA determination with a four-year interval (1996-1999, 2000-2003 and 2004-2007), except for men aged 67 years at the first screening, who were invited only to two screening rounds. If the PSA concentration was ≥ 4.0 ug/l (or 3.0-3.99 ug/l if free to total PSA ratio was ≤ 16%), men were referred to a local urology clinic for DRE, TRUS and biopsy. During the first three years of the study, DRE was used instead of free to total PSA ratio since it was not on clinical use. (Kilpelainen et al. 2013). Detected cancers were treated according to existing guidelines, similarly to cancers in the control arm.

A total of 2,702 men were excluded from the screening arm due to prostate cancer diagnosis before randomization or during the first year after the last screening round, emigration or death. A total of 4,847 men participated once, 6,958 men participated twice and 9,886 men participated three times to the screening, and there were 7,607 (26.0%) nonparticipants in the screening arm (Pakarainen et al. 2016). A total number of 2,416 (10.2%) prostate cancers were diagnosed from men attending screening (23,771 men) whereas 467 (5.8%) and 3,337 (6.9%) prostate cancers were diagnosed among never-participants of the screening arm (8,095 men) and in the control arm (48,278 men), respectively, during the median follow-up of 12 years (Time from the first of January in the year of randomization ending at death, emigration, or at the common closing date, December 31st, 2010). A total of 149 (0.5%) men in the control arm and 266 (0.6%) men in the screening arm died due to prostate cancer (Kilpelainen et al. 2013).

4.1.5 Statistics Finland

Statistics Finland is the national statistical institution. It collects and maintains data on different aspects of the society. For example, Statistics Finland produces annual statistics on causes of death. The statistics are based on information from the compulsory death certificates, which are coded and complemented with data on deaths from the Population Register. The 10th revision of the International Classification of Diseases (ICD-10) has been used since 1996.

Information on cause of death from Statistics Finland has been found to be reliable (Lahti and Penttila 2001; Lahti and Penttila 2003). A cause-of-death committee evaluated all deaths among FinRSPC participants with prostate cancer between 1996-2003. The decision by the committee based on patient files was compared with the official causes of death from Statistics Finland and concordance was high (97.7%, kappa = 0.95) (Mäkinen et al. 2008). Similar results were observed

in a more recent study; concordance was high with the registry both in the screening arm and in the control arm (94.6%, kappa = 0.88 and 95.4%, kappa = 0.91, respectively) (Kilpelainen et al. 2016).

4.1.6 Care Register for Health Care

The Care Register for Health Care is a register maintained by the National Institute for Health and Welfare. It contains information from 1969 to the present. At first, the register contained information only on inpatient care. In 1998, the register started to collect data on outpatient care from specialized health care and the gathering was expanded to primary health care at the beginning of 2011. The subject of the register is to accumulate information on the actions of healthcare departments for research and statistic. The Care Register collects data on all service periods both at public and private hospitals, the statistical unit being a visit. In addition, the register involves information on patients discharged from inpatient care, number of patients in inpatient care at the end of the year, day surgeries and outpatient care in all public and private hospitals. The basic data include information on patients' national identification number, place of residence, date, type and route of admission, diagnoses, external cause, type of accident and date of discharge, for example.

4.2 Study settings

Study details are presented in Table 9.

4.2.1 Case-control study (I)

Every Finnish man with prostate cancer diagnosed for the first time in 1995-2002 was identified from the Finnish Cancer Registry, altogether 25,029 prostate cancer cases. A total number of 24,723 matched controls without prostate cancer were individually selected from the Population Register Center using incidence density sampling, which means that the selection of controls is determined by the diagnoses of cases. When a case is diagnosed, a control is selected from other members of the cohort having not the diagnosis but being at risk to have it (Greenland and Thomas 1982). The matching was based on the same age (±1 year) and area of residence at the time of the diagnosis. However, no matched control in the same

residence area was found for 121 cases in the oldest age group and hence, they were excluded from the analyses. A total of 963 men in the control group developed prostate cancer during the study period. Therefore, they were included in the analysis twice; first as a control and afterwards as a prostate cancer case. A total of 24,657 case-control pairs were included in the final analyses.

Information on the primary site of cancer, date, method of diagnosis and tumor histology were obtained from the Cancer Registry. Diagnosis was based on histology from the primary tumor in 99.3% of cases. Other methods of diagnosis were clinical diagnosis (0.4%), radiological finding (0.3%) and specific laboratory finding (0.02%). Information on diagnosis method was missing from 185 prostate cancer cases and they were excluded from the analyses. Stage (local, locally advanced, advanced) of prostate cancer was available for 13,616 patients (55% of cases) and 73% of these were localized. There was no information on PSA concentration or Gleason score in the Cancer Registry.

We used the Finnish Prescription Register of SII to gather information on medication use. Prescribed antiarrhythmic agent purchases between 1995 and 2002 were obtained from the database. We were able to link the data from the prescription database to study participants via unique national identification number issued for all Finnish residents. Antiarrhythmic drugs included were quinidine, disopyramide, mexiletine, tocainide, propafenone and flecainide (Na^+-channel inhibitors), amiodarone and sotalol (K^+-channel inhibitors), pindolol, propranolol, timolol, carvedilol, labetalol, oxprenolol, alprenolol and sotalol (non-selective BBs), acebutolol, atenolol, betaxolol, bisoprolol, metoprolol and celiprolol (β1-selective BBs). BBs were also categorized as hydrophilic (sotalol, atenolol and celiprolol) and hydrophobic (rest of analyzed BBs) agents. In addition, digoxin and etilefrine were included. Use of other medication (NSAID, aspirin, antidiabetic medication, statin, antihypertensive, 5-ARI and alpha-blocker) was gathered to evaluate possible effect of comorbidities.

4.2.2 Cohort studies (II-IV)

Men in the FinRSPC screening trial formed the study population in our cohort studies. In studies II and IV, all men who we were able to link with SII prescription database (78,615 men) were used. Of them, 30,194 belonged to the screening arm and 48,421 to the control arm. Each man was followed until the date of prostate cancer diagnosis, emigration, death or common closing date in the study II and until

the date of death, emigration or common closing date in the study IV. In the prostate cancer survival study (III), the study population consisted of 6,537 prostate cancer cases diagnosed in 1996-2009 (3,668 from the screening arm and 2,869 from the control arm). Each man was followed until the date of death, emigration or common closing date in the study III.

There were 6,639 prostate cancer diagnoses before 2010. Of them, 2,584 (42.5%) were detected through screening and 1,938 (31.9%) between the screening rounds. A total of 327 (5.4%) of the cases were among men invited to screening but not participating. The detection method was known for 6,527 cases (98.3%). Majority of the cases was histologically confirmed (97.9%). Other diagnostic methods were clinical diagnosis (0.3%), autopsy (1.6%), radiological finding (0.1%) and cytological finding (0.1%).

Information on the Gleason grade at diagnosis, TNM stage, primary treatment, the date and method of diagnosis and the serum PSA concentration was included in the FinRSPC study. We used the criteria of the European Association of Urology to categorized prostate cancer cases to low/medium risk or high risk. Medication besides antiarrhythmic agents was used to evaluate comorbidities. We had data on NSAID, aspirin, antidiabetic medication, statin, antihypertensive, 5-ARI and alpha-blocker use. In addition, data on socioeconomic and demographic factors such as marriage and level of education was gathered from the Statistics Finland. Furthermore, information on the tumor characteristics (such as the Gleason grade and TNM stage) was collected individually from hospitals' patient records for the control arm.

We obtained causes of death in the study population during 1996–2015 from the Statistics Finland. In the survival study (III), cases were recorded as a prostate cancer death if prostate cancer (ICD-10 code C61) was recorded as the primary cause of death. Deaths with ICD-10 code C61 as the intermediate or contributory cause of death were analyzed separately for PCa-related mortality. In the mortality study (IV), cases with prostate (C61), lung (C34), colorectal (C18), pancreatic (C25), gastric (C16), liver (C22), renal (C64), non-Hodgkin lymphoma (C81), bladder (C67) or central nervous system cancer (C71 and C72) recorded as the primary cause of death were considered as cancer deaths. Overall cancer mortality included deaths with any C-code in ICD-10 (C00-C99) as the primary cause of death.

We collected information on diagnoses recorded for in- and outpatient hospital contacts during 1996–2012 from the Care Register for Health Care. The data was used to calculate a modified CCI for the study population (Quan et al. 2011).

Furthermore, we gathered information on antiarrhythmic drug use indications: heart failure (ICD-10 code I50) and cardiac arrhythmias (I47 and I49).

Data on medication use was gathered from the prescription database of the SII. All antiarrhythmic agents purchased during 1995–2015 were obtained and linked to the study population by the national identification number. Agents categorized as antiarrhythmic drugs included amiodarone, digoxin, disopyramide, etilefrine, flecainide, quinidine, mexiletine, procainamide, propafenone and sotalol.

4.3 Statistical methods

4.3.1 Case-control study (I)

We collected information on medication use of prostate cancer cases from the beginning of 1995 to prostate cancer diagnosis. Similarly, medication use was followed from 1995 to the date of diagnosis of the matched case among the controls. To equalize the dose information of antiarrhythmic medication use between different drug groups, we divided the purchased milligram amount of a drug by its DDD set by the World Health Organization (WHO) (WHO Collaborating Centre for Drug Statistics Methodology). DDDs contained in each purchase for a drug were summed up to obtain a total sum of DDDs of every antiarrhythmic agent used by each study participant.

Conditional logistic regression models were used to estimate OR and 95% confidence intervals (CI) for ORs. Overall prostate cancer rate and advanced prostate cancer rate related to antiarrhythmic medication were calculated. We performed both age-adjusted analyses (age at diagnosis, treated as a continuous variable) and analyses further adjusted for use of other medications. Non-users of antiarrhythmic drugs were the reference group in all analyses. Initially, we also included place of residence in the regression model. However, it was not statistically non-significant predictor of prostate cancer risk due to matching of case-control pairs and was therefore left out of the study analyses.

In addition to evaluation of association between ever-use of antiarrhythmic medication and prostate cancer risk, we performed analyses stratified by quartiles of the amount, duration and intensity of medication use. Annual amount of medication use was estimated by calculating the cumulative milligram amount from every purchase during each year separately for all antiarrhythmic agents. The cumulative

amount was standardized by dividing the milligram amount of each drug with the drug-specific standard DDD. Duration of antiarrhythmic drug use was calculated by adding together the number of years with medication purchases. Finally, the average intensity of use (DDDs/year) was calculated by dividing the annual DDD amount with the duration of usage. Men discontinuing use before the end of follow-up retained the level reached and continued from that level if purchases reoccurred.

Trends in association between prostate cancer risk and antiarrhythmic drug use were analyzed by adding cumulative number of DDDs or years of drug use into the logistic regression model as a continuous variable. We analyzed all antiarrhythmic drugs separately and in broader groups (all antiarrhythmic drugs, Na^+-channel inhibitors, K^+-channel inhibitors, β1-selective and non-selective BBs).

Several sensitivity analyses were performed. We included 5alpha-reductase inhibitor and alpha-blocker users only in the analysis to estimate the effect of previous PSA-testing. Furthermore, we gathered data on use of other drug groups during the follow-up from the prescription database of the SII to evaluate possible effect modification. The analysis was stratified by use of cholesterol-lowering medication, NSAIDs, antidiabetic drugs and antihypertensive drugs. Statistical significance of interaction between digoxin and each drug mentioned above was evaluated by including an interaction term (e.g. antiarrhythmic drug use*statin use) in the logistic regression model and p-values less than 0.05 were considered statistically significant.

The data were analyzed using Stata 8.2 software (College Station, Texas).

4.3.2 Cohort studies (II-IV)

We considered antiarrhythmic drug use as a time-dependent variable. Medication use status was updated annually throughout the follow-up based on medication purchases obtained from the prescription database. All participants were categorized as non-users until the first medication purchase and after the first purchase, the exposure status changed to user. If a user had no antiarrhythmic drug purchases during a calendar year, the status was updated into previous user. However, it was possible to change the status back to a user if a further drug purchase occurred at a later point of the follow-up. We used non-users as the reference group in studies II and IV, except in the study III, where users of other antiarrhythmic drugs other than digoxin and sotalol were the reference group.

The survival study (III) differed from the other two analyses. Use of antiarrhythmic medication before prostate cancer diagnosis was analyzed as a time-independent variable fixed at baseline. Men with antiarrhythmic drug use within the year preceding the diagnosis were classified as active users. If a man used antiarrhythmic medication before but not during the calendar year of diagnosis, he was classified as a previous user. In addition, we combined active users and previous users into any use category. Use of antiarrhythmic drugs after the prostate cancer diagnosis was analyzed as a time-dependent variable with the principles mentioned above.

Cox regression method was used in all studies. Various model adjustments were used. All studies included age-adjusted analyses (age as a continuous variable). In addition, the multivariable model in the study estimating prostate cancer risk (II) included adjustments for age, screening trial arm and use of NSAIDs, aspirin, antidiabetic medication, cholesterol-lowering medication, antihypertensives, 5alpha-reductase inhibitors and alpha-blockers. Information on use of other medication during the follow-up was gathered from the prescription database of the SII. In the survival study (III), we used a regression model adjusted for age and EAU tumor risk group and a multivariable-adjusted model similar to the model mentioned above. The study on overall cancer mortality included analyses with adjustment for baseline cancer diagnosis, trial arm (when prostate cancers were considered) and use of other drug groups.

Amount, duration and intensity of medication use were calculated similarly as described in the chapter 4.3.1. Trends in prostate cancer risk, prostate cancer survival and cancer mortality by amount, duration and intensity of used medication were estimated by generating a new exposure variable in which the cohort was stratified by the median (in the study III) or tertiles (in the studies II and IV) of the cumulative amount, duration and intensity.

In the incidence study (II), we analyzed prostate cancer risk overall and by Gleason grade and cancer stage. Subgroup analyses were conducted by stratifying the population by age, antiarrhythmic medication use before randomization and by use of other drug groups. Effect modification on prostate cancer risk among antiarrhythmic drug users was evaluated by adding an interaction term with medication use into the Cox regression model.

In the study on prostate cancer survival (III), we evaluated risk of prostate cancer death among men using digoxin or sotalol compared to the users of other antiarrhythmic drugs. Analyses were conducted separately for pre- and post-diagnostic antiarrhythmic drug use. We stratified the study population according to

age, tumor characteristics, screening trial arm, use of other drug groups and primary treatment and performed subgroup analyses to evaluate effect modification. In the subgroup analyses, non-users were the reference group. In the sensitivity analyses, we estimated delayed effects of drug use by conducting a lag time analysis by relating the drug use to outcomes occurring 1-3 years later. Furthermore, a competing risk analysis with deaths from other causes than cancer as the competing events was performed using the method described by Fine and Gray (Fine and Gray 1999).

In the overall cancer mortality study (IV), we estimated the association of antiarrhythmic drug use with both risk of cancer death overall and deaths due to specific types of cancer. Mortality was evaluated among users of any antiarrhythmic drugs and separately among digoxin and sotalol users. In subgroup analyses, effect modification by age, baseline cancer, use of other drug groups and socioeconomic factors was estimated by stratifying men according to the variables mentioned above. In addition, we conducted similar exposure lagging and competing risk regression analyses with non-cancer deaths as the competing risk as in the prostate cancer survival study (III).

All statistical tests are two-sided. No adjustment for multiple testing was employed. P values 0.05 or less were considered statistically significant. IBM SPSS Statistics 22 (Chicago, IL, USA) software was used in the studies II and III and IBM SPSS Statistics 23 (Chicago, IL, USA) in the study IV for data analyses.

Table 9. Summary of study characteristics

Study	Study setting	Study period	Data sources	Study population	Exposure variables	Exposure period
I	Case-control	1995-2002	Finnish Cancer Registry Population Register Center SII prescription register	24,657 (12,328 case-control pairs)	Any use	1995-2002
II	Cohort study	1996-2012	FinRSPC Population Register Center Finnish Cancer Registry SII prescription register	78,615 men	Current user Previous user	1995-2009
III	Cohort study	1996-2012	FinRSPC Population Register Center Finnish Cancer Registry SII prescription register Statistics Finland	6,537 men with prostate cancer	Any use Current user Previous user	1995-2009
IV	Cohort study	1996-2015	FinRSPC Population Register Center Finnish Cancer Registry SII prescription register Statistics Finland Care Register for Health Care	78,615 men	Any use	1995-2015

Study	Outcomes	Follow-up period	Confounding variables	Time of determination of confounders	Effect modifiers
I	Prostate cancer diagnosis Advanced prostate cancer diagnosis	1995-2002	Age, use of antihypertensive drugs, cholesterol-lowering medication, antidiabetic drugs, NSAIDs, 5-ARIs and alpha-blockers	1995-2002	-
II	Prostate cancer diagnosis Gleason 7-10 prostate cancer diagnosis Advanced prostate cancer diagnosis	1996-2012	Age, screening trial arm, use of cholesterol-lowering medication, antidiabetic drugs, antihypertensive drugs, NSAIDs, 5-ARIs and alpha-blockers	1995-2009	Screening trial arm
III	Prostate cancer death	1996-2012	Age, screening trial arm, use of cholesterol-lowering medication, antidiabetic drugs, antihypertensive drugs, NSAIDs, 5-ARIs and alpha-blockers	1995-2009	-
IV	Overall cancer death Death by separate cancer types	1996-2015	Age, screening trial arm, cancer diagnosis at baseline, use of cholesterol-lowering medication, antidiabetic drugs, antihypertensive drugs, NSAIDs, 5-ARIs and alpha-blockers	1995-2015	Antidiabetic drug use Antihypertensive drug use CCI

SII = Social Insurance Institution of Finland, FinRSPC = Finnish Randomized Study of Screening for Prostate Cancer, NSAID = Non-steroidal anti-inflammatory drugs, 5-ARI = 5-alpha-reductase inhibitors, CCI = Charlson Comorbidity Index

4.4 Ethical considerations

Ethical considerations in prostate cancer screening are related to PSA testing and its poor sensitivity and specificity. Even though prostate cancer screening might improve survival, a great number of men need to be screened and prostate cancers to be detected to avoid one prostate cancer death. Prostate biopsy might cause adverse effects and side effects can be related to prostate cancer treatment methods. It is possible that harmless asymptomatic prostate cancer is diagnosed by PSA screening and the patient suffers a permanent adverse effect from the cancer treatment.

The ethics committee of the Pirkanmaa health care district reviewed the protocol of the case-control study (tracking number ETL R03290). According to the Finnish regulations, it was not necessary to obtain informed consent from the study population, as the analysis was based on data routinely gathered to national registries and on already gathered FinRSPC data, and as part of the study cohort was deceased or emigrated.

The ethical committees of Helsinki University Hospital and Tampere University Hospital had evaluated the FinRSPC trial protocol (tracking number R10167). Informed consent was received from the participants in the screening arm. Men in the control arm were not contacted and were followed via national registries.

Privacy and data protection; all personal identifiers were deleted from the data used in the analyses and results reported in such form that no individuals can be identified, as all health-related information is regarded as sensitive and confidential.

5 RESULTS

5.1 Antiarrhythmic drug use and risk of prostate cancer

5.1.1 Case-control study (I)

The number of antiarrhythmic drug users was similar among cases and control; 3,408 men (13.8%) among the cases and 3,316 men (13.4%) among the controls. Prevalence of use for NSAIDs (53.8% vs 46.5%), cholesterol-lowering medication (10.6% vs 9.9%), antihypertensives (51.6% vs 47.6%) and BPH medication (18.7% vs 12.5%), was higher among the cases than controls. However, there were fewer antidiabetic medication users (9.0% vs 9.7%).

Compared to non-users, overall antiarrhythmic drug use was associated neither with the overall prostate cancer risk (OR 0.96, 95% CI 0.91-1.01) nor advanced prostate cancer risk (OR 0.90, 95% CI 0.77-1.04) (Table 10). There were no significant association between prostate cancer risk and antiarrhythmic drug use within any quartile of cumulative amount and duration of drug use. However, some suggestion of a decreasing trend in overall prostate cancer risk by duration of usage was observed (p for trend = 0.058).

There was no association between digoxin use and overall prostate cancer risk (OR 0.96, 95% CI 0.90-1.02). Similarly, risk of advanced prostate cancer risk did not differ by digoxin use (OR 0.89, 95% CI 0.76-1.05) (Table 10). Digoxin use was not associated with prostate cancer risk in analysis stratified by quartiles of digoxin usage. There was no statistically significant trend in prostate cancer risk by cumulative duration of digoxin use.

Neither use of Na^+-channel blockers nor K^+-channel blockers as a group was associated with prostate cancer risk (OR 0.98, 95% CI 0.84-1.13 and OR 0.94, 95% CI 0.85-1.03, respectively). A similar observation was made for advanced prostate cancer risk (Table 10). There was no association between use of any individual Na^+-channel blocker and prostate cancer risk. However, sotalol users had a statistically significant risk decrease for advanced prostate cancer compared to non-users (OR 0.73, 95% CI 0.56-0.96) (Table 10). Even though the overall prostate cancer risk did

not differ from non-users (OR 0.94, 95% CI 0.85-1.04), it was diminished among long-term (5 years or longer) sotalol users (OR 0.68, 95% CI 0.55-0.85). There was a significant decreasing trend in overall prostate cancer risk by duration of sotalol use (p for trend = 0.038).

Neither β1-selective nor non-selective BBs associated with prostate cancer risk. (OR 1.00, 95% CI 0.96-1.05 and OR 0.99, 95% CI 0.93-1.06, respectively). However, there was an association between hydrophilic BBs and advanced prostate cancer (OR 0.80, 95% CI 0.68-0.96). After exclusion of sotalol, the association between hydrophilic BBs and the risk of advanced prostate cancer disappeared (OR 0.95, 95% CI 0.78-1.16) (Table 10).

Table 10. Prostate cancer risk of antiarrhythmic drug users compared to non-users among 24,657 Finnish prostate cancer cases and matched controls during 1995-2002.

	Overall prostate cancer			Advanced disease[a]		
Drug type	N of exposed cases	OR (95% CI) age-adjusted	OR (95% CI) multivariable-adjusted[b]	N of exposed cases	OR (95% CI) age-adjusted	OR (95% CI) multivariable-adjusted[b]
Any antiarrhythmic drug	3,408	1.03 (0.98-1.09)	0.96 (0.91-1.01)	465	0.92 (0.80-1.05)	0.90 (0.77-1.04)
Digoxin	2,616	1.03 (0.97-1.09)	0.96 (0.90-1.02)	375	0.92 (0.79-1.07)	0.89 (0.76-1.05)
Na^+-channel blockers	378	1.06 (0.92-1.23)	0.98 (0.84-1.13)	39	0.78 (0.51-1.19)	0.76 (0.50-1.18)
Quinidine	207	1.04 (0.85-1.26)	0.94 (0.77-1.15)	22	0.73 (0.41-1.27)	0.73 (0.41-1.31)
Disopyramide	93	1.11 (0.82-1.49)	1.04 (0.77-1.40)	14	1.56 (0.68-3.61)	1.73 (0.74-4.05)
Mexiletine	44	1.05 (0.69-1.60)	0.93 (0.61-1.43)	5	0.71 (0.23-2.25)	0.61 (0.19-1.97)
Propafenone	47	1.42 (0.91-2.22)	1.21 (0.77-1.91)	4	0.80 (0.21-2.98)	0.55 (0.15-2.06)
Flecainide	84	1.22 (0.89-1.68)	1.05 (0.76-1.45)	4	0.44 (0.14-1.44)	0.35 (0.10-1.15)
K^+-channel blockers	858	1.01 (0.92-1.12)	0.94 (0.85-1.03)	103	0.78 (0.60-1.01)	0.78 (0.60-1.02)
Amiodarone	99	1.05(0.79-1.40)	0.99 (0.74-1.32)	11	1.57 (0.61-4.05)	1.74 (0.66-4.57)

Sotalol	823	1.02 (0.93-1.13)	0.94 (0.85-1.04)	98	0.73 (0.56-0.96)	0.73 (0.56-0.96)
Beta-blockers						
β1-selective[c]	6,516	1.06 (1.02-1.10)	1.00 (0.96-1.05)	844	0.99 (0.64-1.79)	1.00 (0.88-1.12)
Non-selective[d]	2,370	1.04 (0.98-1.10)	0.99 (0.93-1.06)	296	0.85 (0.72-1.00)	0.85 (0.72-1.00)
w/o sotalol	1,646	1.06 (0.99-1.14)	1.03 (0.96-1.11)	211	0.95 (0.78-1.16)	0.95 (0.78-1.16)
Hydrophilic[e]	2,284	1.04 (0.98-1.11)	1.00 (0.93-1.06)	272	0.81 (0.68-0.96)	0.80 (0.68-0.96)
w/o sotalol	1,537	1.04 (0.97-1.12)	1.01 (0.93-1.08)	183	0.86 (0.70-1.05)	0.84 (0.68-1.04)
Other:						
Etilefrine	133	0.87 (0.69-1.10)	0.80 (0.63-1.01)	13	0.72 (0.35-1.48)	0.66 (0.32-1.37)

[a] Includes all stage T3 and T4, N+ and M+ tumors

[b] Calculated with conditional logistic regression adjusted for age and use of antihypertensive drugs, cholesterol-lowering medication, antidiabetic drugs, NSAIDs, 5-ARIs and alpha-blockers

[c] Includes acebutolol, atenolol, betaxolol, bisoprolol, metoprolol and celiprolol

[d] Includes sotalol, pindolol, propranolol, timolol, carvedilol, labetalol, oxprenolol and alprenolol

[e] Includes sotalol, atenolol and celiprolol

5.1.2 Cohort study (II)

There were 8,064 (10.3%) antiarrhythmic drug users, 5,668 (7.2%) digoxin users and 2,540 (3.2%) sotalol users. Baseline PSA levels did not differ by antiarrhythmic drug use. A total of 6,639 prostate cancer cases were diagnosed during the median follow-up of 12 years. Prevalence of use for NSAIDs (82.0% vs 78.9%), aspirin (18.4% vs 15.4%), 5-ARIs (14.2% vs 12.1%), alpha-blockers (31.8% vs 26.6%), antihypertensive drugs (96.1% vs 64.0%), antidiabetic drugs (30.3% vs 19.1%) and cholesterol-lowering medication (53.7% vs 40.4%) was higher among the antiarrhythmic drug users compared to the non-users.

Compared to non-users, antiarrhythmic drug users had a slightly elevated overall prostate cancer risk in the age-adjusted analysis (HR 1.13, 95% CI 1.01-1.27). However, the association was reduced in the multivariable-adjusted analysis (HR 1.05, 95% CI 0.94-1.18) (Table 11). The risks of high-grade and metastatic prostate cancer were analyzed separately, and no significant risk difference was observed (HR 0.90, 95% CI 0.74-1.08 and HR 1.21, 95% CI 0.80-1.83, respectively). There were no significant trends in prostate cancer risk by cumulative amount, duration or intensity of antiarrhythmic drug use.

Ever use of digoxin was not associated with prostate cancer risk (HR 1.01, 95% CI 0.87-1.16). A similar observation was made for high-grade cancer and metastatic disease (Table 11). No statistically significant trends in prostate cancer were observed by amount, duration or intensity of digoxin use, even though risk estimates for high-grade and advanced disease tended to decrease with increasing amount and duration. Among men using digoxin for ≥6 years, the HR of high-grade prostate cancer was 0.71 (95% CI 0.49-1.03). In the subgroup analysis, men in the screening arm had a decreased risk for high-grade prostate cancer if they had used digoxin for longer than 5 years (HR 0.31, 95% CI 0.12-0.84).

There was no association between sotalol use and prostate cancer (HR 0.97, 95% CI 0.76-1.24). Sotalol had no influence on risk of high-grade or advanced prostate cancer, either (Table 11). Prostate cancer risk did not differ by amount, duration or intensity of sotalol use.

The association between antiarrhythmic drugs and prostate cancer risk was modified by age at randomization. A non-significantly lowered risk estimate by antiarrhythmic drug use was observed among men aged 55-59 years at baseline (HR 0.84, 95% CI 0.68-1.04), while no risk difference was observed among men aged 63-67 (HR 1.09, 95% CI 0.95-1.25) (p for interaction = 0.001). Sotalol use was

associated with a decreased overall prostate cancer risk among men aged 55-59 years at entry (HR 0.54, 95% CI 0.30-0.97 and p for interaction = 0.006). There was no effect modification by use of other medications.

Table 11. Prostate cancer risk, overall and by grade and stage of antiarrhythmic drug users compared to non-users in the FinRSPC.

		All FinRSPC men		Screening arm	Control arm
	N of men	Age-adjusted analysis	Multivariable-adjusted analysis[a]	Multivariable-adjusted analysis[a,b]	Multivariable-adjusted analysis[a,b]
Overall prostate cancer risk		HR (95% CI)	HR (95% CI)	HR (95% CI)	HR (95% CI)
All antiarrhythmic drugs					
Current users	319	1.13 (1.01-1.27)	1.05 (0.94-1.18)	0.97 (0.81-1.16)	1.09 (0.94-1.26)
Previous users	197	1.08 (0.93-1.26)	1.00 (0.86-1.17)	1.00 (0.78-1.28)	0.99 (0.81-1.21)
Digoxin					
Current users	191	1.06 (0.92-1.23)	1.01 (0.87-1.16)	0.82 (0.64-1.04)	1.13 (0.94-1.35)
Previous users	135	1.09 (0.90-1.31)	1.03 (0.85-1.24)	1.08 (0.81-1.43)	0.97 (0.76-1.25)
Sotalol					
Current users	63	1.05 (0.82-1.34)	0.97 (0.76-1.24)	0.88 (0.60-1.30)	1.05 (0.76-1.45)
Previous users	129	1.16 (0.96-1.41)	1.07 (0.88-1.29)	1.13 (0.84-1.54)	1.02 (0.80-1.30)
Gleason 7-10 prostate cancer risk					

All antiarrhythmic drugs					
Current users	118	0.99 (0.82-1.19)	0.90 (0.74-1.08)	0.92 (0.67-1.25)	0.88 (0.70-1.11)
Previous users	100	1.17 (0.95-1.44)	1.06 (0.86-1.31)	1.23 (0.87-1.74)	0.97 (0.74-1.26)
Digoxin					
Current users	73	0.94 (0.75-1.19)	0.87 (0.69-1.10)	0.67 (0.43-1.04)	0.97 (0.73-1.27)
Previous users	66	1.12 (0.86-1.46)	1.04 (0.80-1.35)	1.28 (0.85-1.93)	0.91 (0.64-1.29)
Sotalol					
Current users	25	1.11 (0.75-1.65)	1.03 (0.69-1.52)	1.25 (0.69-2.26)	0.91 (0.54-1.54)
Previous users	57	1.10 (0.83-1.46)	1.00 (0.75-1.33)	1.40 (0.90-2.16)	0.81 (0.56-1.18)
Metastatic prostate cancer risk[c]					
All antiarrhythmic drugs					
Current users	24	1.33 (0.88-2.01)	1.21 (0.80-1.83)	1.48 (0.71-3.07)	1.10 (0.66-1.84)
Previous users	12	1.03 (0.55-1.93)	0.94 (0.50-1.77)	0.74 (0.18-3.02)	1.00 (0.49-2.03)
Digoxin					
Current users	15	1.29 (0.77-2.16)	1.14 (0.68-1.92)	1.06 (0.39-2.90)	1.18 (0.64-2.16)
Previous users	11	1.39 (0.72-2.70)	1.25 (0.64-2.44)	1.02 (0.25-4.18)	1.34 (0.63-2.85)

Sotalol					
Current users	6	1.55 (0.69-3.46)	1.49 (0.67-3.35)	1.83 (0.45-7.43)	1.36 (0.50-3.65)
Previous users	5	0.87 (0.36-2.11)	0.83 (0.34-2.01)	2.23 (0.70-7.10)	0.42 (0.10-1.68)

a From Cox regression model adjusted for age, screening trial arm and use of cholesterol-lowering medication, antidiabetic and antihypertensive drugs, aspirin and other NSAIDs, and 5alpha-reductase inhibitors and alpha-blockers

b p for interaction in risk of prostate cancer among digoxin users by FinRSPC study arm = 0.052

c Stage M1 at diagnosis

FinRSPC = Finnish Randomized Study for Screening of Prostate Cancer

5.2 Antiarrhythmic drug use and prostate cancer survival (III)

Among 6,537 prostate cancer cases, 730 men (11.2%) had used antiarrhythmic drugs, 485 (7.4%) digoxin and 241 (3.7%) sotalol after the randomization. In total 1,861 men died during the median follow-up of 7.5 years after PCa diagnosis. There were 617 deaths (9.4%) with prostate cancer as the underlying cause of death. Prevalence of use for aspirin (15.8% vs 13.3%), 5-ARIs (14.2% vs 13.8%), alpha-blockers (49.7% vs 46.0%), antihypertensive drugs (97.8% vs 69.5%), antidiabetic drugs (27.7% vs 18.5%) and cholesterol-lowering medication (57.3% vs 45.5%) was higher among the antiarrhythmic drug users compared to the non-users.

Pre-diagnostic digoxin use had no effect on prostate cancer survival compared to users of other antiarrhythmic drugs (HR 1.00, 95% CI 0.56-1.80 and HR 1.21, 95% CI 0.71-2.05 for current use and any use, respectively) (Table 12). Stratifying the analysis over the median of cumulative amount, duration or intensity of pre-diagnostic digoxin use increased HR of prostate cancer death compared to users of other antiarrhythmic drugs but the associations were statistically non-significant (HR 1.56, 95% CI 0.84-2.91, HR 1.54, 95% CI 0.77-3.05 and HR 1.32, 95% CI 0.71-2.47, respectively).

There was no obvious association between post-diagnostic digoxin use and prostate cancer survival (HR 0.81, 95% CI 0.43-1.51 and HR 1.00, 95% CI 0.59-1.71 for current use and any use, respectively) (Table 12). The risk of prostate cancer death was decreased statistically non-significantly if the cumulative amount (HR 0.59, 95% CI 0.24-1.43) or duration (HR 0.31, 95% CI 0.08-1.17) of post-diagnostic digoxin use was above the median.

Pre-diagnostic sotalol use was not associated with prostate cancer survival (HR 0.82, 95% CI 0.34-1.97) (Table 12). Similarly, post-diagnostic use of sotalol was not associated with the risk of prostate cancer death (HR 0.80, 95% CI 0.25-2.26), but men who had discontinued sotalol use had an increased risk of prostate cancer death (HR 2.73, 95% CI 1.28-5.84) compared to the users of other antiarrhythmic drugs. Risk estimates tended to decrease with cumulative sotalol amount and duration of use, but the CIs remained wide.

Compared to non-users, overall antiarrhythmic drug use before prostate cancer diagnosis did not associate with prostate cancer survival (HR 1.16, 95% CI 0.82-1.65). An analogous observation was made for men with any antiarrhythmic drug use after the diagnosis (HR 0.94, 95% CI 0.61-1.44). Furthermore, we compared

digoxin users to non-users of antiarrhythmic drugs. There was no association between digoxin use before (HR 1.22, 95% CI 0.87-1.72) or after (HR 1.09, 95% CI 0.72-1.65) the diagnosis and prostate cancer survival. Digoxin users had an increased all-cause mortality (HR 1.43, 95% CI 1.08-1.88 and HR 1.46, 95% CI 1.11-1.92, for pre- and post-diagnostic use, respectively).

Table 12. Prostate cancer-specific survival among men using digoxin and sotalol compared to other antiarrhythmic drug users in the cohort of 6,537 prostate cancer cases diagnosed in the Finnish Randomized Study of Prostate Cancer Screening.

	Digoxin			Sotalol		
Pre-diagnostic use	Age-adjusted HR (95% CI)	Multivariable-adjusted1[a] HR (95% CI)	Multivariable-adjusted2[b] HR (95% CI)	Age-adjusted HR (95% CI)	Multivariable-adjusted1[a] HR (95% CI)	Multivariable-adjusted2[b] HR (95% CI)
None	Ref	Ref	Ref	Ref	Ref	Ref
Any	1.33 (0.99-1.77)	1.38 (0.86-2.22)	1.21 (0.71-2.05)	1.07 (0.80-1.43)	1.04 (0.62-1.75)	1.12 (0.63-1.98)
Current user	1.53 (0.88-2.65)	1.33 (0.76-2.31)	1.00 (0.56-1.80)	0.93 (0.40-2.16)	0.80 (0.34-1.87)	0.82 (0.34-1.97)
Previous user	1.69 (0.92-3.11)	1.46 (0.79-2.69)	1.57 (0.84-2.95)	1.17 (0.64-2.11)	1.12 (0.66-2.17)	1.16 (0.63-2.15)
Post-diagnostic use						
None	Ref	Ref	Ref	Ref	Ref	Ref
Any	1.19 (0.72-1.97)	1.14 (0.69-1.88)	1.00 (0.59-1.71)	1.56 (0.83-2.92)	1.35 (0.72-2.53)	1.53 (0.78-2.98)

Current user	1.02 (0.60-1.87)	0.95 (0.52-1.74)	0.81 (0.43-1.51)	0.73 (0.23-2.34)	0.67 (0.21-2.15)	0.80 (0.25-2.64)
Previous user	1.62 (0.78-3.36)	1.62 (0.79-3.36)	1.42 (0.64-3.18)	2.56 (1.24-5.29)	2.08 (1.00-4.32)	2.73 (1.28-5.84)

[a] From Cox regression model adjusted for age and tumor risk group

[b] From Cox regression model adjusted for age, screening trial arm and use of cholesterol-lowering medication, antidiabetic and antihypertensive drugs, aspirin and other NSAIDs, and 5alpha-reductase inhibitors and alpha-blockers and additionally for tumor risk group

5.3 Antiarrhythmic drug use and cancer mortality (IV)

In the study cohort of 78,615 men, there were 9,023 (11.5%) antiarrhythmic drug users, 6,329 (8.1%) digoxin users and 2,304 (2.9%) sotalol users. A total of 28,936 men died, 8,889 (%) of them from cancer during the median follow-up of 17.0 years. The most frequent cancers were lung cancer (2,384 deaths), colorectal cancer (861 deaths) and pancreatic cancer (782 deaths). Prevalence for use of NSAIDs (82.5% vs 78.8%), aspirin (18.3% vs 15.4%), alpha-blockers (32.2% vs 26.5%), antihypertensive drugs (93.7% vs 63.9%), antidiabetic drugs (28.5% vs 19.1%) and cholesterol-lowering medication (53.6% vs 40.3%) was higher and the CCI was higher (CCI 2 or greater 41.1% vs 23.1%) among antiarrhythmic drug users compared to non-users.

Ever-users of antiarrhythmic drug had an increased risk of cancer death compared to the non-users (HR 1.43, 95% CI 1.34-1.53). A comparable observation was made for users of digoxin and sotalol (HR 1.59, 95% CI 1.47-1.72 and HR 1.16, 95% CI 1.03-1.31, respectively) (Table 13). The risk of death decreased with increasing amount, duration and intensity of any antiarrhythmic drug use and the risk association disappeared among men with largest cumulative amount and duration of medication use. A comparable observation was made for digoxin use. In addition, risk estimates attenuated in the lag-time analysis (Table 13).

Overall antiarrhythmic drug use and digoxin use were both associated with increased risk of lung cancer death compared to the non-users (HR 1.72, 95% CI 1.52-1.95 and HR 2.10, 95% CI 1.82-2.41, respectively). However, there was no association between sotalol use and lung cancer mortality (HR 1.10, 95% CI 0.85-1.41) (Table 13). Analogous trends in risk difference by amount, duration and intensity as with overall cancer mortality were observed.

Both users of antiarrhythmic drugs in general and users of digoxin had an increased colorectal cancer mortality compared to non-users (HR 1.38, 95% CI 1.11-1.73 and HR 1.59, 95% CI 1.24-2.05, respectively). Sotalol use was not associated with the risk for colorectal cancer death (HR 0.70, 95% CI 0.42-1.17) (Table 13).

Antiarrhythmic drug use had no effect on pancreatic cancer mortality (HR 1.02, 95% CI 0.79-1.31). Neither use of digoxin nor sotalol associated with the risk of pancreatic cancer death (HR 1.06, 95% CI 0.79-1.43 and HR 0.99, 95 % CI 0.63-1.54, respectively) (Table 13).

Use of antihypertensives and use of antidiabetic medication modified the association between antiarrhythmic drugs and cancer mortality. Among non-users of antihypertensives, men with overall antiarrhythmic drug use or with digoxin use had more substantial increase in the risk of cancer death than among men using antihypertensives (p for interaction 0.01 and 0.002, respectively). A similar phenomenon was also seen for antidiabetic medication usage (p for interaction 0.01).

CCI was used to stratify the study population by comorbidities. Among men without major comorbidities, antiarrhythmic drug users had an increased cancer mortality (CCI 0: HR 1.37, 95% CI 1.19-1.56). Similarly, antiarrhythmic drug use was associated with non-significant increase in cancer mortality among men with intermediate comorbidities (CCI 1: HR 1.22, 95% CI 0.87-1.71), but the result was statistically non-significant. However, there was no association between antiarrhythmic drug use and the risk of cancer death among men with most comorbidities (CCI 2 or greater: 0.98, 95% CI 0.91-1.06). CCI was a statistically significant effect modifier (p for interaction < 0.001).

Antiarrhythmic drug use was not associated with cancer mortality in competing risk analyses (HR 1.04, 95% CI 0.97–1.12). Similar results were obtained for use of digoxin and sotalol (HR 1.01, 95% CI 0.93–1.10 and HR 1.03, 95% CI 0.91–1.17, respectively).

The risk association between the indications for antiarrhythmic drug use and cancer mortality were used to evaluate possible confounding by indication. In the Care Register for Health Care database, 4,199 men had a diagnosis of cardiac insufficiency and 1,507 men had a diagnosis of arrhythmia. Men with a diagnosis of cardiac insufficiency had increased cancer mortality compared to the men without the diagnosis (HR 1.19, 95% CI 1.08-1.31). The risk increase was comparable to the result in our main analysis. However, having a recorded diagnosis of arrhythmia was associated with a lowered risk of cancer death (HR 0.76, 95% CI 0.64-0.90).

Table 13. Antiarrhythmic drug use and cancer mortality in Finnish Randomized Study of Screening for Prostate Cancer.

	Overall cancer death[a]		Lung cancer death	Colorectal cancer death	Pancreatic cancer death
	Age-adjusted model	Multivariable-adjusted model[b]	Multivariable-adjusted model[c]	Multivariable-adjusted model[c]	Multivariable-adjusted model[c]
Antiarrhythmic drug use	HR (95%CI)	HR (95%CI)	HR (95%CI)	HR (95%CI)	HR (95%CI)
No use	Ref	Ref	Ref	Ref	Ref
Any use	1.40 (1.31-1.50)	1.43 (1.34-1.53)	1.72 (1.52-1.95)	1.38 (1.11-1.73)	1.02 (0.79-1.31)
Lag 3 years	1.24 (1.15-1.34)	1.26 (1.17-1.36)	1.39 (1.20-1.61)	1.36 (1.07-1.74)	0.98 (0.74-1.30)
Lag 5 years	1.21 (1.12-1.31)	1.23 (1.13-1.33)	1.29 (1.10-1.51)	1.42 (1.10-1.82)	0.99 (0.74-1.33)
Digoxin use					
No use	Ref	Ref	Ref	Ref	Ref
Any use	1.60 (1.48-1.73)	1.59 (1.47-1.72)	2.10 (1.82-2.41)	1.59 (1.24-2.05)	1.06 (0.79-1.43)
Lag 3 years	1.35 (1.23-1.47)	1.33 (1.21-1.45)	1.59 (1.34-1.88)	1.53 (1.15-2.02)	1.00 (0.72-1.40)
Lag 5 years	1.30 (1.18-1.44)	1.28 (1.16-1.41)	1.49 (1.23-1.79)	1.59 (1.19-2.14)	0.97 (0.67-1.39)
Sotalol use					
No use	Ref	Ref	Ref	Ref	Ref
Any use	1.11 (0.98-1.25)	1.16 (1.03-1.31)	1.10 (0.85-1.41)	0.70 (0.42-1.17)	0.99 (0.63-1.54)

Lag 3 years	1.11 (0.98-1.26)	1.16 (1.02-1.32)	1.07 (0.82-1.39)	0.83 (0.51-1.37)	0.98 (0.61-1.57)
Lag 5 years	1.08 (0.95-1.24)	1.14 (0.99-1.30)	0.98 (0.74-1.31)	0.89 (0.54-1.47)	1.06 (0.66-1.69)

[a] Including lung, prostate, colorectal, pancreatic, gastric, liver, renal, non-Hodgkin lymphoma, bladder and central nervous system cancer

[b] From Cox regression model adjusted for age, screening trial arm and use of cholesterol-lowering medication, antidiabetic and antihypertensive drugs, aspirin and other NSAIDs, 5alpha-reductase inhibitors, alpha-blockers and cancer diagnose at baseline

[c] From Cox regression model adjusted for age and use of cholesterol-lowering medication, antidiabetic and antihypertensive drugs, aspirin and other NSAIDs, 5alpha-reductase inhibitors, alpha-blockers and cancer diagnose at baseline

6 DISCUSSION

6.1 Antiarrhythmic drugs and risk of prostate cancer

In the case-control study, antiarrhythmic medication use did not have an influence on prostate cancer risk at population level. Nevertheless, sotalol use was associated with a decreased risk of advanced prostate cancer. Furthermore, men with five or more years with sotalol use had a lower overall prostate cancer risk compared to non-users.

In the cohort study, there was no association between prostate cancer risk and use of antiarrhythmic medication, digoxin or sotalol, but there was a decreasing trend for Gleason 7-10 cancer risk with increasing duration of digoxin use. However, users of any antiarrhythmic drug had a comparable decreasing trend in high-grade cancer risk by duration of use, and digoxin did not have the protective association with high-grade prostate cancer risk when compared to other antiarrhythmic drug users. Therefore, our observation might be due to systematic differences between users and non-users of antiarrhythmic drugs rather than due to pharmacological properties of digoxin. Nonetheless, we observed a borderline non-significant risk reduction among digoxin users in the screening arm. The risk reduction was statistically significant for Gleason 7-10 cancers among long-term users of digoxin in the screening arm. However, screening of prostate cancer did not modify risk association between digoxin use and Gleason 7-10 cancer statistically significantly (p for interaction = 0.33).

Our results are partly coherent with previously published studies. In The Health Professionals Follow-up study of 47,884 men, digoxin users had a decreased risk of overall prostate cancer (RR 0.78, 95% CI 0.67-0.90) compared to the non-users. The association was strongest for men with 10 or more years of digoxin use (RR 0.54, 95% CI 0.37-0.79) (Platz et al. 2011). Since PSA testing is rather common in the US, the study population of the Health Professionals Follow-up study was probably in regular PSA surveillance. In the Europe, PSA testing has been more infrequent compared to the US, but men in the screening study were under regular PSA testing making the screening arm more comparable to the US population. Thus, there is no

inconsistency between our results and those from The Health Professionals Follow-up study.

A previous case-control study with 1,943 men observed decreased risk of prostate cancer among digoxin users but association was statistically non-significant association (OR 0.58, 95% CI 0.30-1.10). Contrary to our studies, long-term users had a more prominent increase in the risk estimates than short-term users. However, digoxin users with 3 or more PSA-tests during the past five years had a decreased prostate cancer risk (OR 0.44, 95% CI 0.20-0.98) (Wright et al. 2014). This finding is coherent with our result of digoxin users under regular PSA-surveillance having lower prostate cancer risk than men in the control arm.

In conclusion, PSA surveillance is a modifying factor for digoxin users' prostate cancer risk and the results suggest that digoxin users attending regular PSA-surveillance have a slightly lowered prostate cancer risk compared to the non-users. It has been suggested that digoxin might decrease PSA-concentration which could explain the observed finding (Lin et al. 2014). Especially long-term use of digoxin might be beneficial as antineoplastic effects likely require long-term exposure to occur. A protective association between sotalol use and prostate cancer risk was observed in the case-control but not in the cohort study.

6.2 Antiarrhythmic drugs and prostate cancer survival

In the prostate cancer survival study, prostate cancer survival among digoxin or sotalol users did not differ from men using other antiarrhythmic drugs. The results were similar for both pre- and postdiagnostic use. Stratifying analyses by cumulative amount, duration or intensity of medication use did not change the results although long-term users of digoxin had lower risk estimates than short-term users. The trial arm (screening vs control), tumor characteristics, screening, use of other drug groups and primary treatment did not modify the results.

Our study is in concordance with two previous studies on digoxin use and prostate cancer survival. Flavahan et al. followed 5,732 prostate cancer cases for a median follow-up of 4.3 years. The full study cohort had 391 men with digoxin exposure before the prostate cancer diagnosis. Propensity score matched sensitivity analysis with 387 digoxin-exposed and 387 non-exposed men was performed. In the full cohort, digoxin users had an increased all-cause mortality (HR 1.24, 95% CI 1.07-1.43), but there was no statistically significant increase in prostate cancer-specific mortality (HR 1.13, 95% CI 0.91-1.42). The results were comparable in the

propensity score matched population (HR 1.20, 95% CI 1.00-1.49 for all-cause mortality and HR 1.17, 95% CI 0.88-1.57 for prostate cancer-specific mortality). The influence of the cumulative duration of digoxin use was not analyzed. (Flahavan et al. 2014).

Karesneh et al. evaluated prostate cancer survival among 13,134 prostate cancer cases for the mean follow-up of 5.0 years. Of them, 701 (5.3%) had used digoxin after prostate cancer diagnosis. In the adjusted analysis (adjusted for year of diagnosis, age at diagnosis, cancer treatment within 6 months, comorbidities prior to diagnosis and other medication use), digoxin use was not associated with prostate cancer-specific survival (HR 1.13, 95% CI 0.93-1.37). However, digoxin users had increased all-cause mortality compared to the non-users (HR 1.39, 95% CI 1.23-1.56). Stratifying the analysis by cumulative amount did not affect to the risk estimates and the influence of the duration of digoxin use was not evaluated (Karasneh et al. 2016). Analyses are comparable to our survival study; we had a parallel percentage of digoxin users within the cohort (7.4%) and the adjustments were coherent.

Especially long-term use of digoxin has been reported to have anticancer effects, and since we were able to stratify the study population by duration of digoxin use, our study provides additional information to the prior studies. Furthermore, we evaluated the effect of both pre- and post-diagnostic digoxin use, contributing novel information on the effect of the timing of medication use.

In conclusion, we did not observe statistically significant risk reductions among digoxin or sotalol users. Our results and previously published studies suggest that digoxin use does not improve prostate cancer survival.

6.3 Antiarrhythmic drugs and cancer mortality

In study IV, antiarrhythmic drug users had a statistically significantly increased overall cancer mortality and lung cancer mortality compared to non-users. Furthermore, digoxin use was associated with a statistically significantly increased risk of death from all cancers combined, lung cancer, colorectal cancer, bladder cancer and non-Hodgkin lymphoma. There was no statistically significant association between sotalol use and risk of cancer death in the age-adjusted analysis. However, in the multivariable analysis sotalol users had an increased cancer mortality similar to digoxin users indicating sotalol users had less risk factors for cancer death.

The observed elevation in risk of cancer death among antiarrhythmic drug users is likely due to differences between users and non-users. We observed a similar risk association between indication of digoxin use (cardiac insufficiency) and cancer mortality. In addition, the association tended to decrease with increasing cumulative amount and duration of antiarrhythmic medication. An opposite trend would probably be observed if antiarrhythmic medication increased the risk directly. Therefore, the association between drug use and cancer mortality is unlikely to be due to pharmacological properties of antiarrhythmic medication, but more probably due to confounding by systematic background differences, such as smoking, alcohol consumption and high-fat diet, between antiarrhythmic drug users and non-users. Digoxin users have more co-morbidities, such as coronary artery disease, congestive heart failure and diabetes mellitus, than non-users, which might result in a non-causal risk association in our study. A subgroup analysis stratified by the CCI supports this. Among men with no major co-morbidity burden (CCI = 0), digoxin users had a statistically significant increase in cancer mortality. However, among men with more co-morbidities (CCI = 1 and CCI ≥ 2 or more), the risk association was no observed indicating that the association is modified by co-morbidities. Furthermore, the CCI was found to be an independent risk factor for cancer death and in the competing risk analysis, where non-cancer deaths were the competing risk, antiarrhythmic drug use was not associated with risk of cancer death. Antiarrhythmic drug use could not be considered as a time-dependent variable, and thus is possible that immortal time bias is not as robust controlled as in Cox regression analysis leading to disappearance of risk increase.

Our results are coherent with previous studies on digoxin but there are no previous publications on other antiarrhythmic drug use and an overall cancer mortality. There are a few published studies on use of digoxin and cancer mortality in separate cancer types and a meta-analysis based on the studies. Digoxin use was associated with a statistically significantly increased all-cause mortality (HR 1.35, 95% CI 1.25-1.46), but cancer-specific mortality did not differ between digoxin users and non-users (HR 1.08, 95% CI 0.97-1.19) in the meta-analysis of six cohort studies (Osman et al. 2017).

In conclusion, antiarrhythmic drug use did not decrease cancer mortality in our retrospective study. In contrast, antiarrhythmic drug users had an increased cancer mortality compared to non-users, but the risk association was probably non-causal, since it was found mainly in short-term use and vanished in long-term use. Possible antineoplastic effects of digoxin or any other antiarrhythmic drug did not translate to a diminished cancer mortality.

6.4 Methodological considerations

We used two study populations; the case-control study containing all prostate cancer cases diagnosed in Finland during 1995-2002 and the study population from the FinRSPC. Both study settings had different strengths and limitations allowing a thorough evaluation of the research question.

Both studies were population-based with a solid representation of the entire Finland. The case-control study had a population of 49,314 men and cohort studies 78,615 men and the prostate cancer survival analysis covered 6,537 prostate cancer cases from the FinRSPC population. The Finnish Cancer Registry is a robust source of information since the coverage of cancer diagnosed in Finland is comprehensive. Therefore, misclassification of outcome and related information bias are likely to be minimal. Large study populations enabled statistical power to analyze rather seldom used drugs. Nevertheless, CIs for especially subgroup analyses were wide in the survival study. One possible limitation is potential indolent prostate cancers diagnosed in men the FinRSPC screening arm. However, since there were both antiarrhythmic drug users and non-users in the screening arm, confounding is unlikely and furthermore, we were able to analyze separately high-grade cancer risk and prostate cancer-specific survival.

Since we obtained information on drug purchases from the prescription database, surveys on medication exposure were not required and recall bias does not affect the results. Furthermore, data on cancer treatment and clinical characteristics was gathered from medical records allowing us to adjust analysis by cancer grade and stage or by given treatment. In the case-control study, the controls were individually matched to the cases for age and residential area at the time of diagnosis to avoid confounding by the above-mentioned variables.

We updated medication usage status annually and use of antiarrhythmic drugs was treated as a time-dependent variable in order to avoid immortal time bias. Men were classified as non-users before the first antiarrhythmic purchase occurred. Failing to consider unexposed time period correctly in the regression analysis will result in immortal time bias. Immortal time refers to a time interval during the follow-up where it would be impossible for the outcome to occur. Immortal time bias often occurs if a time period prior to initiating an exposure of interest (antiarrhythmic drug use in this study) is not assessed properly. Since outcome cannot occur during the time span, it is inevitable that the subject remains free of outcome until the exposure has truly initiated if classified as exposed (Suissa 2008).

We used different sensitivity analyses to evaluate influence of biases and confounding and to evaluate possible causality. The total propensity score for use of antiarrhythmic medication was calculated and the analysis for prostate cancer risk was stratified by quartiles or median of the total propensity score in order to estimate the risk in subgroups homogenous in possible background confounding factors. The delayed effects of medication use were estimated by conducting a lag time analysis in which we related the drug use to outcomes occurring 1-5 years later.

When the event of interest is cancer death, competing risks might modify the chance that the outcome happens. In order to evaluate the cumulative incidence function, which indicates the probability of outcome occurring before a given time, we performed competing risk regression analyses with non-cancer deaths as the competing risk using the method described by Fine and Gray (Fine and Gray 1999). We performed the competing risk regression, where non-cancer deaths were competing risk, since antiarrhythmic drug users might die due to vascular diseases which would bias down risk estimates for cancer mortality. Surprisingly, risk of cancer death was lower in the competing risk regression compared to the Cox regression suggesting that deaths due to non-cancer causes did not bias down the risk estimates. CCI was used to stratify the study population by comorbidities and to estimate risk of outcomes among men with different range of comorbid conditions.

Confounding by indication means that observed association between an exposure (antiarrhythmic medication) and an outcome (prostate cancer risk or death) is not due to the exposure but an indication or a factor related to the indication for which the exposure was used (Salas, Hofman, Stricker 1999). Smoking is a risk factor for both heart diseases and cancer. Therefore, especially in the overall cancer mortality study confounding by indication is likely to exist. To evaluate the influence of confounding by indication, users of other antiarrhythmic drugs were used as the reference group for digoxin and sotalol users, thus performing analysis in a group where we assumed everyone had the identical indication for antiarrhythmic drug use. These results were compared to analyses where non-users of antiarrhythmic drugs were the reference group. In addition, we used antihypertensive drug users as the reference group, since they are frequently used in the management of cardiac insufficiency, a common indication also for digoxin use. Furthermore, antiarrhythmic drug users' risk of death due to any cause was calculated since digoxin is used in management of atrial fibrillation and cardiac insufficiency, which are associated with cardiovascular diseases. The risk association between the indication for digoxin use (cardiac insufficiency or arrhythmias) and cancer mortality was observed.

Several limitations need to be considered. We gathered the information on medication use from the national prescription database, which records the reimbursed drug purchases. There is no certainty whether men have used the medication they bought, which might have caused exposure misclassification. Since exposure status might be equally misclassified among men developing and not developing the outcome, misclassification is non-differential. Non-differential misclassification biases the results towards the null if the exposure variable is dichotomous (Dosemeci, Wacholder, Lubin 1990; Wacholder et al. 1995). In addition, we have no information on medication used during inpatient stays. Data on antiarrhythmic drug use before 1995 was not available which may lead to underestimation of cumulative amount and duration of drug use among men with history of antiarrhythmic drug use prior to 1995 resulting, probably in bias towards the null, assuming information bias is non-differential. We had no information on use of over-the-counter drugs but since purchase of any antiarrhythmic agent requires a prescription in Finland, this limitation does not cause bias in our study. We gathered information on drug use during 1995-2015 so current deductible did not affect our study. However, deductible prior to 2005 might have caused some bias since all digoxin purchases were not necessarily reimbursed; if a patient bought only digoxin, the total cost might have remained under 10 euros and the purchase was not recorded into the prescription database. The limitations mentioned above might fade the potential protective effect of digoxin use. In the study III we compared digoxin users to users of other antiarrhythmic drugs which increases possibility of immortal time bias. Nevertheless, digoxin users were compared to non-users in sensitivity analyses and comparable results were obtained indicating immortal time bias is unlikely.

There was no information on behavioral factors such as smoking, diet, ejaculation frequency or family history which might have caused confounding. Smoking is a strong risk factor for cardiac diseases, and it worsens cancer patients' prognosis and thus it might have caused confounding increasing observed risk estimates among medication users. Men using antiarrhythmic medication might be sexually less active, and thus ejaculate less frequently. This might bias results away from the null since it has been observed that high ejaculation frequency is associated with a decreased prostate cancer risk. Furthermore, we did not have data on the number of health care contacts which might have been greater among men with antiarrhythmic medication. This might result in earlier detection of tumor, more active treatment and lower cancer mortality creating so called healthy user bias. We performed a

variety of subgroup analyses and therefore, multiple comparison bias might exist resulting in an erroneous statistically significant finding.

Users and non-users of antiarrhythmic drugs are not necessarily comparable, since medication users have often more comorbidity compared to non-users. This might result in non-causal risk differences in epidemiological studies and provides a likely explanation for the observed increase in cancer mortality among antiarrhythmic drug users. CCI was an independent risk factor for cancer death and in subgroup analyses stratified by CCI, we observed an increased cancer mortality among digoxin users without major comorbidity (CCI 0), but there was no risk difference between digoxin users and non-users among men with comorbidity (CCI>0). In addition, use of antiarrhythmic medication was not associated with risk of cancer death in the competing risk analyses providing additional evidence for the hypothesis that use of antiarrhythmic drugs is not associated with cancer mortality when the effect of non-cancer deaths as competing causes are taken into consideration.

6.5 Future considerations

Even though digoxin has been a promising antineoplastic agent in *in vitro* experiments and there are several plausible pharmacological mechanisms to suggest cancer protective effects, digoxin use has not been consistently associated with a diminished cancer incidence or an improved cancer-specific survival. However, long-term digoxin use of more than 10 years might be beneficial especially against prostate cancer. Very long-term benefits might exist. In future, it might be justifiable to evaluate effects of digoxin at population level with extended follow-up (20 years for example). The ideal study population should have comparable comorbidities and baseline characteristics, since it is inadequate to compare men using digoxin to healthy men. In addition, information on antiarrhythmic drug use should be as accurate as possible. Clinical trials comparing digoxin to other antiarrhythmic drugs in order to test cancer protective effects are not indicated currently, since short-term impact of digoxin has not been observed on epidemiological studies.

Sotalol users had a reduced risk of advanced prostate cancer in our case-control study. However, use of sotalol did not associate with risk of prostate cancer, prostate cancer survival or overall cancer mortality in the FinRSPC cohort. As

epidemiological evidence of effects of sotalol is uncertain, the priority of future studies is to define mechanism of possible beneficial effect of sotalol in *in vitro* studies in order to find out which target group would be most likely to benefit.

7 CONCLUSION

This study shows that use of digoxin does not increase prostate cancer incidence, worsen prostate cancer prognosis or increase overall mortality. The main limitation of our study was possible differences in baseline characteristics and comorbidities, and therefore users and non-users of antiarrhythmic drugs were not necessarily comparable. Very long-term use of digoxin might be beneficial due to antineoplastic effects of digoxin, but the effect is probably minor, since it was not clearly observed in our large study population during the relatively long follow-up of over 10 years. Use of other antiarrhythmic medication is neither advantageous nor harmful when considering prostate cancer.

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9 REFERENCES

Agalliu I, Kwon EM, Zadory D, McIntosh L, Thompson J, Stanford JL, Ostrander EA. 2007. Germline mutations in the BRCA2 gene and susceptibility to hereditary prostate cancer. Clinical Cancer Research. 13(3):839-43.

Allan C, Ilic D. 2016. Laparoscopic versus robotic-assisted radical prostatectomy for the treatment of localised prostate cancer: A systematic review. Urol Int. 96(4):373-8.

Allemani C, Matsuda T, Di Carlo V, Harewood R, Matz M, Niksic M, Bonaventure A, Valkov M, Johnson CJ, Esteve J, et al. 2018. Global surveillance of trends in cancer survival 2000-14 (CONCORD-3): Analysis of individual records for 37 513 025 patients diagnosed with one of 18 cancers from 322 population-based registries in 71 countries. Lancet. 391(10125):1023-75.

Andriole GL, Bostwick DG, Brawley OW, Gomella LG, Marberger M, Montorsi F, Pettaway CA, Tammela TL, Teloken C, Tindall DJ, et al. 2010. Effect of dutasteride on the risk of prostate cancer. N Engl J Med. 362(13):1192-202.

Applegate CC, Rowles JL, Ranard KM, Jeon S, Erdman JW. 2018. Soy consumption and the risk of prostate cancer: An updated systematic review and meta-analysis. Nutrients. 10(1):10.3390/nu10010040.

Arsov C, Rabenalt R, Blondin D, Quentin M, Hiester A, Godehardt E, Gabbert HE, Becker N, Antoch G, Albers P, et al. 2015. Prospective randomized trial comparing magnetic resonance imaging (MRI)-guided in-bore biopsy to MRI-ultrasound fusion and transrectal ultrasound-guided prostate biopsy in patients with prior negative biopsies. Eur Urol. 68(4):713-20.

Auvinen A, Rannikko A, Taari K, Kujala P, Mirtti T, Kenttamies A, Rinta-Kiikka I, Lehtimaki T, Oksala N, Pettersson K, et al. 2017. A randomized trial of early detection of clinically significant prostate cancer (ProScreen): Study design and rationale. Eur J Epidemiol. 32(6):521-7.

Batta K, Das C, Gadad S, Shandilya J, Kundu TK. 2007. Reversible acetylation of non histone proteins: Role in cellular function and disease. Subcell Biochem. 41:193-212.

Beer TM, Armstrong AJ, Rathkopf DE, Loriot Y, Sternberg CN, Higano CS, Iversen P, Bhattacharya S, Carles J, Chowdhury S, et al. 2014. Enzalutamide in metastatic prostate cancer before chemotherapy. N Engl J Med. 371(5):424-33.

Bell KJ, Del Mar C, Wright G, Dickinson J, Glasziou P. 2015. Prevalence of incidental prostate cancer: A systematic review of autopsy studies. Int J Cancer. 137(7):1749-57.

Berger AP, Gozzi C, Steiner H, Frauscher F, Varkarakis J, Rogatsch H, Bartsch G, Horninger W. 2004. Complication rate of transrectal ultrasound guided prostate biopsy: A comparison among 3 protocols with 6, 10 and 15 cores. J Urol. 171(4):147-1.

Bhardwaj A, Srivastava SK, Khan MA, Prajapati VK, Singh S, Carter JE, Singh AP. 2017. Racial disparities in prostate cancer: A molecular perspective. Frontiers in Bioscience (Landmark Edition). 22:772-82.

Bill-Axelson A, Holmberg L, Ruutu M, Garmo H, Stark JR, Busch C, Nordling S, Haggman M, Andersson SO, Bratell S, et al. 2011. Radical prostatectomy versus watchful waiting in early prostate cancer. N Engl J Med. 364(18):1708-17.

Bolla M, Van Tienhoven G, Warde P, Dubois JB, Mirimanoff RO, Storme G, Bernier J, Kuten A, Sternberg C, Billiet I, et al. 2010. External irradiation with or without long-term androgen suppression for prostate cancer with high metastatic risk: 10-year results of an EORTC randomised study. Lancet Oncol. 11(11):1066-73.

Bonkhoff H. 2018. Estrogen receptor signaling in prostate cancer: Implications for carcinogenesis and tumor progression. Prostate. 78(1):2-10.

Brawley OW. 2012. Prostate cancer epidemiology in the united states. World J Urol. 30(2):195-200.

Bray F, Ferlay J, Soerjomataram I, Siegel RL, Torre LA, Jemal A. 2018. Global cancer statistics 2018: GLOBOCAN estimates of incidence and mortality worldwide for 36 cancers in 185 countries. CA Cancer J Clin. 68(6):394-424.

Bubendorf L, Schopfer A, Wagner U, Sauter G, Moch H, Willi N, Gasser TC, Mihatsch MJ. 2000. Metastatic patterns of prostate cancer: An autopsy study of 1,589 patients. Hum Pathol. 31(5):578-83.

Cao L, Zhang S, Jia CM, He W, Wu LT, Li YQ, Wang W, Li Z, Ma J. 2018a. Antihypertensive drugs use and the risk of prostate cancer: A meta-analysis of 21 observational studies. BMC Urol. 18(1):1-7.

Cao L, Zhang S, Jia CM, He W, Wu LT, Li YQ, Wang W, Li Z, Ma J. 2018b. Antihypertensive drugs use and the risk of prostate cancer: A meta-analysis of 21 observational studies. BMC Urol. 18(1):1-7.

Carlsson S, Assel M, Sjoberg D, Ulmert D, Hugosson J, Lilja H, Vickers A. 2014. Influence of blood prostate specific antigen levels at age 60 on benefits and harms of prostate cancer screening: Population based cohort study. Bmj. 348.

Carruba G. 2007. Estrogen and prostate cancer: An eclipsed truth in an androgen-dominated scenario. J Cell Biochem. 102(4):899-911.

Carter BS, Beaty TH, Steinberg GD, Childs B, Walsh PC. 1992. Mendelian inheritance of familial prostate cancer. Proc Natl Acad Sci U S A. 89(8):3367-71.

Catalona WJ, Partin AW, Sanda MG, Wei JT, Klee GG, Bangma CH, Slawin KM, Marks LS, Loeb S, Broyles DL, et al. 2011. A multicenter study of -2]pro-prostate specific antigen combined with prostate specific antigen and free prostate specific antigen for prostate cancer detection in the 2.0 to 10.0 ng/ml prostate specific antigen range. J Urol. 185(5):1650-5.

Chen N, Zhou Q. 2016. The evolving gleason grading system. Chinese Journal of Cancer Research = Chung-Kuo Yen Cheng Yen Chiu. 28(1):58-64.

Chen YB, Chen Q, Wang Z, Zhou J. 2013. Insulin therapy and risk of prostate cancer: A systematic review and meta-analysis of observational studies. PLoS One. 8(11):e81594.

Chen Y, Chen Q, Wang Z, Zhou J. 2013. Insulin therapy and risk of prostate cancer: A systematic review and meta-analysis of observational studies. Plos One. 8(11):e81594.

Cheng L, Montironi R, Bostwick DG, Lopez-Beltran A, Berney DM. 2012. Staging of prostate cancer. Histopathology. 60(1):87-117.

Choi CH, Song T, Kim TH, Choi JK, Park JY, Yoon A, Lee YY, Kim TJ, Bae DS, Lee JW, et al. 2014. Meta-analysis of the effects of beta blocker on survival time in cancer patients. J Cancer Res Clin Oncol. 140(7):1179-88.

Chou R, Croswell JM, Dana T, Bougatsos C, Blazina I, Fu R, Gleitsmann K, Koenig HC, Lam C, Maltz A, et al. 2011. Screening for prostate cancer: A review of the evidence for the U.S. preventive services task force. Ann Intern Med. 155(11):762-71.

Chung MH, Wang YW, Chang YL, Huang SM, Lin WS. 2017. Risk of cancer in patients with heart failure who use digoxin: A 10-year follow-up study and cell-based verification. Oncotarget. 8(27):44203-16.

Cole SW, Sood AK. 2012. Molecular pathways: Beta-adrenergic signaling in cancer. Clin Cancer Res. 18(5):1201-6.

Cox ME, Gleave ME, Zakikhani M, Bell RH, Piura E, Vickers E, Cunningham M, Larsson O, Fazli L, Pollak M. 2009. Insulin receptor expression by human prostate cancers. Prostate. 69(1):33-40.

Cui Y, Zong H, Yan H, Zhang Y. 2014. The effect of testosterone replacement therapy on prostate cancer: A systematic review and meta-analysis. Prostate Cancer and Prostatic Diseases. 17:132.

Dadaev T, Saunders EJ, Newcombe PJ, Anokian E, Leongamornlert DA, Brook MN, Cieza-Borrella C, Mijuskovic M, Wakerell S, Olama AAA, et al. 2018. Fine-mapping of prostate cancer susceptibility loci in a large meta-analysis identifies candidate causal variants. Nature Communications. 9(1):2256.

Dall'Era MA, Albertsen PC, Bangma C, Carroll PR, Carter HB, Cooperberg MR, Freedland SJ, Klotz LH, Parker C, Soloway MS. 2012. Active surveillance for prostate cancer: A systematic review of the literature. Eur Urol. 62(6):976-83.

de Bono JS, Oudard S, Ozguroglu M, Hansen S, Machiels JP, Kocak I, Gravis G, Bodrogi I, Mackenzie MJ, Shen L, et al. 2010. Prednisone plus cabazitaxel or mitoxantrone for metastatic castration-resistant prostate cancer progressing after docetaxel treatment: A randomised open-label trial. Lancet. 376(9747):1147-54.

de Koning HJ, Gulati R, Moss SM, Hugosson J, Pinsky PF, Berg CD, Auvinen A, Andriole GL, Roobol MJ, Crawford ED, et al. 2018. The efficacy of prostate-specific antigen screening: Impact of key components in the ERSPC and PLCO trials. Cancer. 124(6):1197-206.

Dearnaley DP, Jovic G, Syndikus I, Khoo V, Cowan RA, Graham JD, Aird EG, Bottomley D, Huddart RA, Jose CC, et al. 2014. Escalated-dose versus

control-dose conformal radiotherapy for prostate cancer: Long-term results from the MRC RT01 randomised controlled trial. Lancet Oncol. 15(4):464-73.

Delongchamps NB, Peyromaure M, Schull A, Beuvon F, Bouazza N, Flam T, Zerbib M, Muradyan N, Legman P, Cornud F. 2013. Prebiopsy magnetic resonance imaging and prostate cancer detection: Comparison of random and targeted biopsies. J Urol. 189(2):493-9.

Discacciati A, Orsini N, Wolk A. 2012. Body mass index and incidence of localized and advanced prostate cancer—a dose–response meta-analysis of prospective studies. Annals of Oncology. 23(7):1665-71.

Discacciati A, Wolk A. 2014. Lifestyle and dietary factors in prostate cancer prevention. Recent Results in Cancer Research. 202:27-37.

Doat S, Cenee S, Tretarre B, Rebillard X, Lamy PJ, Bringer JP, Iborra F, Murez T, Sanchez M, Menegaux F. 2017. Nonsteroidal anti-inflammatory drugs (NSAIDs) and prostate cancer risk: Results from the EPICAP study. Cancer Med. 6(10):2461-70.

Dosemeci M, Wacholder S, Lubin JH. 1990. Does nondifferential misclassification of exposure always bias a true effect toward the null value? Am J Epidemiol. 132(4):746-8.

Draisma G, Boer R, Otto SJ, van der Cruijsen, I. W., Damhuis RA, Schroder FH, de Koning HJ. 2003. Lead times and overdetection due to prostate-specific antigen screening: Estimates from the european randomized study of screening for prostate cancer. J Natl Cancer Inst. 95(12):868-78.

Duodecim. 2014. Prostate cancer. current care guidelines. Helsinki: The Finnish Medical Society Duodecim.

Egami K, Murohara T, Shimada T, Sasaki K, Shintani S, Sugaya T, Ishii M, Akagi T, Ikeda H, Matsuishi T, et al. 2003. Role of host angiotensin II type 1 receptor in tumor angiogenesis and growth. J Clin Invest. 112(1):67-75.

Egevad L, Granfors T, Karlberg L, Bergh A, Stattin P. 2002. Prognostic value of the gleason score in prostate cancer. BJU Int. 89(6):538-42.

Epstein JI, Egevad L, Amin MB, Delahunt B, Srigley JR, Humphrey PA. 2016. The 2014 international society of urological pathology (ISUP) consensus conference on gleason grading of prostatic carcinoma definition of grading

patterns and proposal for a new grading system. Am J Surg Pathol. 40(2):244-52.

Ewing CM, Ray AM, Lange EM, Zuhlke KA, Robbins CM, Tembe WD, Wiley KE, Isaacs SD, Johng D, Wang Y, et al. 2012. Germline mutations in HOXB13 and prostate-cancer risk. N Engl J Med. 366(2):141-9.

Fairhurst C, Watt I, Martin F, Bland M, Brackenbury WJ. 2015. Sodium channel-inhibiting drugs and survival of breast, colon and prostate cancer: A population-based study. Sci Rep. 5:16758.

Feng T, Sun X, Howard LE, Vidal AC, Gaines AR, Moreira DM, Castro-Santamaria R, Andriole GL, Freedland SJ. 2015. Metformin use and risk of prostate cancer: Results from the REDUCE study. Cancer Prev Res (Phila). 8(11):1055-60.

Feng Z, Zhou X, Liu N, Wang J, Chen X, Xu X. 2019. Metformin use and prostate cancer risk: A meta-analysis of cohort studies. Medicine (Baltimore). 98(12):e14955.

Ferlay J, Colombet M, Soerjomataram I, Mathers C, Parkin DM, Pineros M, Znaor A, Bray F. 2019. Estimating the global cancer incidence and mortality in 2018: GLOBOCAN sources and methods. Int J Cancer. 144(8):1941-53.

Ferlay J, Soerjomataram I, Dikshit R, Eser S, Mathers C, Rebelo M, Parkin DM, Forman D, Bray F. 2015. Cancer incidence and mortality worldwide: Sources, methods and major patterns in GLOBOCAN 2012. International Journal of Cancer. 136(5):359.

Fine JP, Gray RJ. 1999. A proportional hazards model for the subdistribution of a competing risk. Journal of the American Statistical Association. 94(446):496-509.

Finnish Cancer Registry [Internet]; ca.

Finnish Cancer Registry [Internet]; cb. Available from: http://stats.cancerregistry.fi/stats/fin/vfin0003i0.html .

Finnish Cancer Registry [Internet]; cc. Available from: http://stats.cancerregistry.fi/stats/fin/vfin0005i0.html .

Finnish Cancer Registry
[Internet]; cd. Available from:
http://stats.cancerregistry.fi/stats/fin/vfin0033p0.html .

Finnish Cancer Registry
[Internet]; ce. Available from:
http://stats.cancerregistry.fi/stats/fin/vfin0043p0.html .

Fizazi K, Carducci M, Smith M, Damiao R, Brown J, Karsh L, Milecki P, Shore N, Rader M, Wang H, et al. 2011. Denosumab versus zoledronic acid for treatment of bone metastases in men with castration-resistant prostate cancer: A randomised, double-blind study. Lancet. 377(9768):813-22.

Fizazi K, Scher HI, Molina A, Logothetis CJ, Chi KN, Jones RJ, Staffurth JN, North S, Vogelzang NJ, Saad F, et al. 2012. Abiraterone acetate for treatment of metastatic castration-resistant prostate cancer: Final overall survival analysis of the COU-AA-301 randomised, double-blind, placebo-controlled phase 3 study. Lancet Oncol. 13(10):983-92.

Flahavan EM, Sharp L, Bennett K, Barron TI. 2014. A cohort study of digoxin exposure and mortality in men with prostate cancer. BJU Int. 113(2):236-45.

Freedland SJ, Hamilton RJ, Gerber L, Banez LL, Moreira DM, Andriole GL, Rittmaster RS. 2013. Statin use and risk of prostate cancer and high-grade prostate cancer: Results from the REDUCE study. Prostate Cancer Prostatic Dis. 16(3):254-9.

Furuya Y, Lundmo P, Short AD, Gill DL, Isaacs JT. 1994. The role of calcium, pH, and cell proliferation in the programmed (apoptotic) death of androgen-independent prostatic cancer cells induced by thapsigargin. Cancer Res. 54(23):6167.

Garcia Bennett J, Vilanova JC, Guma Padro J, Parada D, Conejero A. 2017. Evaluation of MR imaging-targeted biopsies of the prostate in biopsy-naive patients. A single centre study. Diagn Interv Imaging. 98(10):677-84.

Gleason DF, Mellinger GT. 1974. Prediction of prognosis for prostatic adenocarcinoma by combined histological grading and clinical staging. J Urol. 111(1):58-64.

Greenland S, Thomas DC. 1982. On the need for the rare disease assumption in case-control studies. Am J Epidemiol. 116(3):547-53.

Grimm P, Billiet I, Bostwick D, Dicker AP, Frank S, Immerzeel J, Keyes M, Kupelian P, Lee WR, Machtens S, et al. 2012. Comparative analysis of prostate-specific antigen free survival outcomes for patients with low, intermediate and high risk prostate cancer treatment by radical therapy. results from the prostate cancer results study group. BJU Int. 109:22-9.

Guazzoni G, Nava L, Lazzeri M, Scattoni V, Lughezzani G, Maccagnano C, Dorigatti F, Ceriotti F, Pontillo M, Bini V, et al. 2011. Prostate-specific antigen (PSA) isoform p2PSA significantly improves the prediction of prostate cancer at initial extended prostate biopsies in patients with total PSA between 2.0 and 10 ng/ml: Results of a prospective study in a clinical setting. Eur Urol. 60(2):214-22.

Gupta S, Srivastava M, Ahmad N, Bostwick DG, Mukhtar H. 2000. Over-expression of cyclooxygenase-2 in human prostate adenocarcinoma. Prostate. 42(1):73-8.

Hackshaw-McGeagh L, Perry RE, Leach VA, Qandil S, Jeffreys M, Martin RM, Lane JA. 2015. A systematic review of dietary, nutritional, and physical activity interventions for the prevention of prostate cancer progression and mortality. Cancer Causes & Control : CCC. 26(11):1521-50.

Hamdy FC, Donovan JL, Lane JA, Mason M, Metcalfe C, Holding P, Davis M, Peters TJ, Turner EL, Martin RM, et al. 2016. 10-year outcomes after monitoring, surgery, or radiotherapy for localized prostate cancer. N Engl J Med. 375(15):1415-24.

Haring A, Murtola TJ, Talala K, Taari K, Tammela TL, Auvinen A. 2017. Antidiabetic drug use and prostate cancer risk in the finnish randomized study of screening for prostate cancer. Scand J Urol. 51(1):5-12.

Harris AL. 2002. Hypoxia—a key regulatory factor in tumour growth. Nature Reviews Cancer. 2(1):38.

Hauswald H, Kamrava MR, Fallon JM, Wang P, Park S, Van T, Borja L, Steinberg ML, Demanes DJ. 2016. High-dose-rate monotherapy for localized prostate cancer: 10-year results. International Journal of Radiation Oncology • Biology • Physics. 94(4):667-74.

Haux J, Klepp O, Spigset O, Tretli S. 2001a. Digitoxin medication and cancer; case control and internal dose-response studies. BMC Cancer. 1:11.

Haux J, Klepp O, Spigset O, Tretli S. 2001b. Digitoxin medication and cancer; case control and internal dose-response studies. BMC Cancer. 1:11.

Health Quality Ontario. 2017. Prolaris cell cycle progression test for localized prostate cancer: A health technology assessment. Ont Health Technol Assess Ser. 17(6):1-75.

Heemsbergen WD, Al-Mamgani A, Slot A, Dielwart MF, Lebesque JV. 2014. Long-term results of the dutch randomized prostate cancer trial: Impact of dose-escalation on local, biochemical, clinical failure, and survival. Radiother Oncol. 110(1):104-9.

Heinonen OP, Koss L, Albanes D, Taylor PR, Hartman AM, Edwards BK, Virtamo J, Huttunen JK, Haapakoski J, Malila N. 1998. Prostate cancer and supplementation with α-tocopherol and β-carotene: Incidence and mortality in a controlled trial. JNCI: Journal of the National Cancer Institute. 90(6):440-6.

Hjelmborg JB, Scheike T, Holst K, Skytthe A, Penney KL, Graff RE, Pukkala E, Christensen K, Adami H, Holm NV, et al. 2014. The heritability of prostate cancer in the nordic twin study of cancer. Cancer Epidemiol Biomarkers Prev. 23(11):2303.

Hoskin PJ, Rojas AM, Bownes PJ, Lowe GJ, Ostler PJ, Bryant L. 2012. Randomised trial of external beam radiotherapy alone or combined with high-dose-rate brachytherapy boost for localised prostate cancer. Radiotherapy and Oncology. 103(2):217-22.

SEER Cancer Statistics Review, 1975-2014 [Internet]; c2016. Available from: https://seer.cancer.gov/csr/1975_2014/ .

Huang H, Cai B. 2014. G84E mutation in HOXB13 is firmly associated with prostate cancer risk: A meta-analysis. Tumour Biology. 35(2):1177-82.

Hughes FM,Jr, Bortner CD, Purdy GD, Cidlowski JA. 1997. Intracellular K+ suppresses the activation of apoptosis in lymphocytes. J Biol Chem. 272(48):30567-76.

Hugosson J, Roobol MJ, Månsson M, Tammela TLJ, Zappa M, Nelen V, Kwiatkowski M, Lujan M, Carlsson SV, Talala KM, et al. A 16-yr follow-up of the european randomized study of screening for prostate cancer. Eur Urol. .

Huncharek M, Haddock KS, Reid R, Kupelnick B. 2010. Smoking as a risk factor for prostate cancer: A meta-analysis of 24 prospective cohort studies. Am J Public Health. 100(4):693-701.

Hwang IC, Park SM, Shin D, Ahn HY, Rieken M, Shariat SF. 2015. Metformin association with lower prostate cancer recurrence in type 2 diabetes: A systematic review and meta-analysis. Asian Pac J Cancer Prev. 16(2):595-600.

Ikonen T, Matikainen MP, Syrjakoski K, Mononen N, Koivisto PA, Rokman A, Seppala EH, Kallioniemi O, Tammela TLJ, Schleutker J. 2003. BRCA1 and BRCA2 mutations have no major role in predisposition to prostate cancer in finland. J Med Genet. 40(8):e98.

Islami F, Moreira DM, Boffetta P, Freedland SJ. 2014. A systematic review and meta-analysis of tobacco use and prostate cancer mortality and incidence in prospective cohort studies. Eur Urol. 66(6):1054-64.

Iversen P, Tyrrell CJ, Kaisary AV, Anderson JB, Van Poppel H, Tammela TL, Chamberlain M, Carroll K, Melezinek I. 2000. Bicalutamide monotherapy compared with castration in patients with nonmetastatic locally advanced prostate cancer: 6.3 years of followup. J Urol. 164(5):1579-82.

Jahn JL, Giovannucci EL, Stampfer MJ. 2015. The high prevalence of undiagnosed prostate cancer at autopsy: Implications for epidemiology and treatment of prostate cancer in the prostate-specific antigen-era. Int J Cancer. 137(12):2795-802.

Jamaspishvili T, Berman DM, Ross AE, Scher HI, De Marzo AM, Squire JA, Lotan TL. 2018. Clinical implications of PTEN loss in prostate cancer. Nat Rev Urol. 15(4):222-34.

Jan CR, Lee KC, Chou KJ, Cheng JS, Wang JL, Lo YK, Chang HT, Tang KY, Yu CC, Huang JK. 2001. Fendiline, an anti-anginal drug, increases intracellular Ca2+ in PC3 human prostate cancer cells. Cancer Chemother Pharmacol. 48(1):37-41.

Jespersen CG, Norgaard M, Friis S, Skriver C, Borre M. 2014. Statin use and risk of prostate cancer: A danish population-based case-control study, 1997-2010. Cancer Epidemiol. 38(1):42-7.

Jian L, Xie LP, Lee AH, Binns CW. 2004. Protective effect of green tea against prostate cancer: A case-control study in southeast china. International Journal of Cancer. 108(1):130-5.

Johns LE, Houlston RS. 2003. A systematic review and meta-analysis of familial prostate cancer risk. BJU Int. 91(9):789-94.

Jousimaa J, Alenius H, Atula S, Berghem N, Kattainen A, Pelttari H, Teikari M. 2017. Lääkärin käsikirja. Kustannus Oy Duodecim.

Kaapu KJ, Murtola TJ, Määttänen L, Talala K, Taari K, Tammela TLJ, Auvinen A. 2016. Prostate cancer risk among users of digoxin and other antiarrhythmic drugs in the finnish prostate cancer screening trial. Cancer Causes & Control. 27(2):157-64.

Kalbasi A, Li J, Berman A, Swisher-McClure S, Smaldone M, Uzzo RG, Small DS, Mitra N, Bekelman JE. 2015. Dose-escalated irradiation and overall survival in men with nonmetastatic prostate cancer. JAMA Oncol. 1(7):897-906.

Kang H, Gillespie TW, Goodman M, Brodie SA, Brandes M, Ribeiro M, Ramalingam SS, Shin DM, Khuri FR, Brandes JC. 2014. Long-term use of valproic acid in US veterans is associated with a reduced risk of smoking-related cases of head and neck cancer. Cancer. 120(9):1394-400.

Kantor ED, Lipworth L, Fowke JH, Giovannucci EL, Mucci LA, Signorello LB. 2015. Statin use and risk of prostate cancer: Results from the southern community cohort study. Prostate. 75(13):1384-93.

Kao LT, Huang CC, Lin HC, Huang CY. 2018. Antiarrhythmic drug usage and prostate cancer: A population-based cohort study. Asian J Androl. 20(1):37-42.

Kao L, Huang C, Lin H, Huang C. 2017. Antiarrhythmic drug usage and prostate cancer: A population-based cohort study. Asian J Androl. 20(1):37-42.

Karasneh RA, Murray LJ, Cardwell CR. 2017. Cardiac glycosides and breast cancer risk: A systematic review and meta-analysis of observational studies. Int J Cancer. 140(5):1035-41.

Karasneh RA, Murray LJ, Hughes CM, Cardwell CR. 2016. Digoxin use after diagnosis of prostate cancer and survival: A population-based cohort study. Pharmacoepidemiol Drug Saf. 25(9):1099-103.

Kasivisvanathan V, Rannikko AS, Borghi M, Panebianco V, Mynderse LA, Vaarala MH, Briganti A, Budäus L, Hellawell G, Hindley RG, et al. 2018. MRI-targeted or standard biopsy for prostate-cancer diagnosis. N Engl J Med. 378(19):1767-77.

Kaufmann S, Kruck S, Kramer U, Gatidis S, Stenzl A, Roethke M, Scharpf M, Schilling D. 2015. Direct comparison of targeted MRI-guided biopsy with systematic transrectal ultrasound-guided biopsy in patients with previous negative prostate biopsies. Urol Int. 94(3):319-25.

Kela, Fimea. 2017. Finnish statistics on medicines 2017. .

Kenfield SA, DuPre N, Richman EL, Stampfer MJ, Chan JM, Giovannucci EL. 2014. Mediterranean diet and prostate cancer risk and mortality in the health professionals follow-up study. Eur Urol. 65(5):887-94.

Kilpelainen TP, Makinen T, Karhunen PJ, Aro J, Lahtela J, Taari K, Talala K, Tammela TL, Auvinen A. 2016. Estimating bias in causes of death ascertainment in the finnish randomized study of screening for prostate cancer. Cancer Epidemiol. 45:1-5.

Kilpelainen TP, Tammela TL, Malila N, Hakama M, Santti H, Maattanen L, Stenman UH, Kujala P, Auvinen A. 2013. Prostate cancer mortality in the finnish randomized screening trial. J Natl Cancer Inst. 105(10):719-25.

Klaukka T. 2009. The finnish database on drug utilisation. Nje. 11(1).

Klein EA, Thompson IM, Tangen CM, Crowley JJ, Lucia MS, Goodman PJ, Minasian LM, Ford LG, Parnes HL, Gaziano JM. 2011. Vitamin E and the risk of prostate cancer: The selenium and vitamin E cancer prevention trial (SELECT). Jama. 306(14):1549-56.

Klotz L, Vesprini D, Sethukavalan P, Jethava V, Zhang L, Jain S, Yamamoto T, Mamedov A, Loblaw A. 2015. Long-term follow-up of a large active surveillance cohort of patients with prostate cancer. J Clin Oncol. 33(3):272-7.

Krieger N, Quesenberry CJ, Peng T, Horn-Ross P, Stewart S, Brown S, Swallen K, Guillermo T, Suh D, Alvarez-Martinez L, et al. 1999. Social class, race/ethnicity, and incidence of breast, cervix, colon, lung, and prostate cancer among asian, black, hispanic, and white residents of the san francisco bay area, 1988-92 (united states). Cancer Causes & Control. 10(6):525-37.

Kuhn JM, Billebaud T, Navratil H, Moulonguet A, Fiet J, Grise P, Louis JF, Costa P, Husson JM, Dahan R. 1989. Prevention of the transient adverse effects of a gonadotropin-releasing hormone analogue (buserelin) in metastatic prostatic carcinoma by administration of an antiandrogen (nilutamide). N Engl J Med. 321(7):413-8.

Kurahashi N, Sasazuki S, Iwasaki M, Inoue M, Shoichiro Tsugane for the JPHC Study Group. 2007. Green tea consumption and prostate cancer risk in japanese men: A prospective study. Am J Epidemiol. 167(1):71-7.

Lahti RA, Penttila A. 2003. Cause-of-death query in validation of death certification by expert panel; effects on mortality statistics in finland, 1995. Forensic Sci Int. 131(2-3):113-24.

Lahti RA, Penttila A. 2001. The validity of death certificates: Routine validation of death certification and its effects on mortality statistics. Forensic Sci Int. 115(1-2):15-32.

Laitinen VH, Wahlfors T, Saaristo L, Rantapero T, Pelttari LM, Kilpivaara O, Laasanen SL, Kallioniemi A, Nevanlinna H, Aaltonen L, et al. 2013. HOXB13 G84E mutation in finland: Population-based analysis of prostate, breast, and colorectal cancer risk. Cancer Epidemiol Biomarkers Prev. 22(3):452-60.

Leinonen MK, Miettinen J, Heikkinen S, Pitkäniemi J, Malila N. 2017. Quality measures of the population-based finnish cancer registry indicate sound data quality for solid malignant tumours. Eur J Cancer. 77:31-9.

Lim YP, Lin CL, Lin YN, Ma WC, Chen WC, Hung DZ, Kao CH. 2015. Antiarrhythmic agents and the risk of malignant neoplasm of liver and intrahepatic bile ducts. PLoS One. 10(1):e0116960.

Lima N, Cavaliere H, Knobel M, Halpern A, Medeiros-Neto G. 2000. Decreased androgen levels in massively obese men may be associated with impaired function of the gonadostat. Int J Obes. 24(11):1433.

Lin J, Zhan T, Duffy D, Hoffman-Censits J, Kilpatrick D, Trabulsi EJ, Lallas CD, Chervoneva I, Limentani K, Kennedy B, et al. 2014. A pilot phase II study of digoxin in patients with recurrent prostate cancer as evident by a rising PSA. American Journal of Cancer Therapy and Pharmacology. 2(1):21-32.

Liss MA, Xu J, Chen H, Kader AK. 2015. Prostate genetic score (PGS-33) is independently associated with risk of prostate cancer in the PLCO trial. Prostate. 75(12):1322-8.

Liu H, Hu GH, Wang XC, Huang TB, Xu L, Lai P, Guo ZF, Xu YF. 2015. Coffee consumption and prostate cancer risk: A meta-analysis of cohort studies. Nutr Cancer. 67(3):392-400.

Liu Y, Hu F, Li D, Wang F, Zhu L, Chen W, Ge J, An R, Zhao Y. 2011. Does physical activity reduce the risk of prostate cancer? A systematic review and meta-analysis. Eur Urol. 60(5):1029-44.

Loblaw DA, Mendelson DS, Talcott JA, Virgo KS, Somerfield MR, Ben-Josef E, Middleton R, Porterfield H, Sharp SA, Smith TJ, et al. 2004. American society of clinical oncology recommendations for the initial hormonal management of androgen-sensitive metastatic, recurrent, or progressive prostate cancer. J Clin Oncol. 22(14):2927-41.

Lonergan PE, Tindall DJ. 2011. Androgen receptor signaling in prostate cancer development and progression. Journal of Carcinogenesis. 10:20; 2-20.

Loriot Y, Massard C, Gross-Goupil M, Di Palma M, Escudier B, Bossi A, Chauchereau A, Fizazi K. 2010. The interval from the last cycle of docetaxel-based chemotherapy to progression is associated with the efficacy of subsequent docetaxel in patients with prostate cancer. Eur J Cancer. 46(10):1770-2.

Magnan S, Zarychanski R, Pilote L, Bernier L, Shemilt M, Vigneault E, Fradet V, Turgeon AF. 2015. Intermittent vs continuous androgen deprivation therapy for prostate cancer: A systematic review and meta-analysisIntermittent vs continuous androgen deprivation for prostate CancerIntermittent vs continuous androgen deprivation for prostate cancer. Oncology. 1(9):1261-9.

Mäkinen T, Karhunen P, Aro J, Lahtela J, Määttänen L, Auvinen A. 2008. Assessment of causes of death in a prostate cancer screening trial. Int J Cancer. 122(2):413-7.

Margel D, Urbach D, Lipscombe LL, Bell CM, Kulkarni G, Austin PC, Fleshner N. 2013. Association between metformin use and risk of prostate cancer and its grade. J Natl Cancer Inst. 105(15):1123-31.

McConkey DJ, Orrenius S. 1997. The role of calcium in the regulation of apoptosis. Biochem Biophys Res Commun. 239(2):357-66.

McConkey DJ, Lin Y, Nutt LK, Ozel HZ, Newman RA. 2000. Cardiac glycosides stimulate ca^{2+} increases and apoptosis in androgen-independent, metastatic human prostate adenocarcinoma cells. Cancer Res. 60(14):3807.

Mohammed N, Kestin L, Ghilezan M, Krauss D, Vicini F, Brabbins D, Gustafson G, Ye H, Martinez A. 2012. Comparison of acute and late toxicities for three modern high-dose radiation treatment techniques for localized prostate

cancer. International Journal of Radiation Oncology • Biology • Physics. 82(1):204-12.

Mottet N, van den Bergh, R. C. N., Briers E, Bourke L, Cornford P, De Santis M, Gillessen S, Govorov A. 2017. EAU guidelines on prostate cancer. .

Mucci LA, Hjelmborg JB, Harris JR, Czene K, Havelick DJ, Scheike T, Graff RE, Holst K, Möller S, Unger RH, et al. 2016. Familial risk and heritability of cancer among twins in nordic CountriesFamilial risk and heritability of cancer among TwinsFamilial risk and heritability of cancer among twins. Jama. 315(1):68-76.

Murtola TJ, Karppa EK, Taari K, Talala K, Tammela TL, Auvinen A. 2016. 5-alpha reductase inhibitor use and prostate cancer survival in the finnish prostate cancer screening trial. Int J Cancer. 138(12):2820-8.

Murtola TJ, Tammela TL, Maattanen L, Huhtala H, Platz EA, Ala-Opas M, Stenman UH, Auvinen A. 2010. Prostate cancer and PSA among statin users in the finnish prostate cancer screening trial. Int J Cancer. 127(7):1650-9.

Murtola TJ, Syvala H, Tolonen T, Helminen M, Riikonen J, Koskimaki J, Pakarainen T, Kaipia A, Isotalo T, Kujala P, et al. 2018. Atorvastatin versus placebo for prostate cancer before radical prostatectomy-A randomized, double-blind, placebo-controlled clinical trial. Eur Urol. 74(6):697-701.

SEER Cancer Statistics Review, 1975-2015 [Internet]; c2017. Available from: https://seer.cancer.gov/csr/1975_2015/ .

Osman MH, Farrag E, Selim M, Osman MS, Hasanine A, Selim A. 2017. Cardiac glycosides use and the risk and mortality of cancer; systematic review and meta-analysis of observational studies. PLoS One. 12(6):e0178611.

Ostrander EA, Udler MS. 2008. The role of the BRCA2 gene in susceptibility to prostate cancer revisited. Cancer Epidemiology, Biomarkers & Prevention. 17(8):1843-8.

O'Sullivan B, Brierley J, D'Cruz A, Fey M, Pollock R, Vermorken J, Hui Huang S. 2015. UICC manual of clinical oncology. Wiley-Blackwell.

Pakarainen T, Raitanen J, Talala K, Taari K, Kujala P, Tammela TL, Auvinen A. 2016. Number of screening rounds and postscreening prostate cancer incidence: Results from the finnish section of the european randomized study of screening for prostate cancer study. Eur Urol. 70(3):499-505.

Parker C, Nilsson S, Heinrich D, Helle SI, O'Sullivan JM, Fossa SD, Chodacki A, Wiechno P, Logue J, Seke M, et al. 2013. Alpha emitter radium-223 and survival in metastatic prostate cancer. N Engl J Med. 369(3):213-23.

Pashayan N, Pharoah PD, Schleutker J, Talala K, Tammela TL, Määttänen L, Harrington P, Tyrer J, Eeles R, Duffy SW, et al. 2015. Reducing overdiagnosis by polygenic risk-stratified screening: Findings from the finnish section of the ERSPC. Br J Cancer. 113(7):1086-93.

Peeters PH, Keinan-Boker L, van der Schouw, Y. T., Grobbee DE. 2003. Phytoestrogens and breast cancer risk. review of the epidemiological evidence. Breast Cancer Res Treat. 77(2):171-83.

Pickles T, Hamm J, Morris WJ, Schreiber WE, Tyldesley S. 2012. Incomplete testosterone suppression with luteinizing hormone-releasing hormone agonists: Does it happen and does it matter? BJU Int. 110(11 Pt B):500.

Pierorazio PM, Walsh PC, Partin AW, Epstein JI. 2013. Prognostic gleason grade grouping: Data based on the modified gleason scoring system. BJU Int. 111(5):753-60.

Pilie PG, Giri VN, Cooney KA. 2016. HOXB13 and other high penetrant genes for prostate cancer. Asian J Androl. 18(4):530-2.

Pinsky PF, Prorok PC, Yu K, Kramer BS, Black A, Gohagan JK, Crawford ED, Grubb RL, Andriole GL. 2017. Extended mortality results for prostate cancer screening in the PLCO trial with median follow-up of 15 years. Cancer. 123(4):592-9.

Platz EA, Rohrmann S, Pearson JD, Corrada MM, Watson DJ, De Marzo AM, Landis PK, Metter EJ, Carter HB. 2005a. Nonsteroidal anti-inflammatory drugs and risk of prostate cancer in the baltimore longitudinal study of aging. Cancer Epidemiol Biomarkers Prev. 14(2):390-6.

Platz EA, Yegnasubramanian S, Liu JO, Chong CR, Shim JS, Kenfield SA, Stampfer MJ, Willett WC, Giovannucci E, Nelson WG. 2011. A novel two-stage, transdisciplinary study identifies digoxin as a possible drug for prostate cancer treatment. Cancer Discov. 1(1):68-77.

Platz EA, Leitzmann MF, Rifai N, Kantoff PW, Chen Y, Stampfer MJ, Willett WC, Giovannucci E. 2005b. Sex steroid hormones and the androgen receptor gene CAG repeat and subsequent risk of prostate cancer in the prostate-specific antigen era. Cancer Epidemiology and Prevention Biomarkers. 14(5):1262-9.

Pokorny MR, de Rooij M, Duncan E, Schroder FH, Parkinson R, Barentsz JO, Thompson LC. 2014. Prospective study of diagnostic accuracy comparing prostate cancer detection by transrectal ultrasound-guided biopsy versus magnetic resonance (MR) imaging with subsequent MR-guided biopsy in men without previous prostate biopsies. Eur Urol. 66(1):22-9.

Population Information System [Internet]. Available from: https://vrk.fi/en/population-information-system .

Prabhu V, Lee T, McClintock TR, Lepor H. 2013. Short-, intermediate-, and long-term quality of life outcomes following radical prostatectomy for clinically localized prostate cancer. Reviews in Urology. 15(4):161-77.

Preston MA, Riis AH, Ehrenstein V, Breau RH, Batista JL, Olumi AF, Mucci LA, Adami HO, Sorensen HT. 2014. Metformin use and prostate cancer risk. Eur Urol. 66(6):1012-20.

Pukkala E, Engholm G, Hojsgaard Schmidt LK, Storm H, Khan S, Lambe M, Pettersson D, Olafsdottir E, Tryggvadottir L, Hakanen T, et al. 2018. Nordic cancer registries - an overview of their procedures and data comparability. Acta Oncol. 57(4):440-55.

Quan H, Li B, Couris CM, Fushimi K, Graham P, Hider P, Januel JM, Sundararajan V. 2011. Updating and validating the charlson comorbidity index and score for risk adjustment in hospital discharge abstracts using data from 6 countries. Am J Epidemiol. 173(6):676-82.

Raval AD, Thakker D, Negi H, Vyas A, Salkini MW. 2016. Association between statins and clinical outcomes among men with prostate cancer: A systematic review and meta-analysis. Prostate Cancer and Prostatic Diseases. 19:151.

Raval AD, Thakker D, Vyas A, Salkini M, Madhavan S, Sambamoorthi U. 2015. Impact of metformin on clinical outcomes among men with prostate cancer: A systematic review and meta-analysis. Prostate Cancer Prostatic Dis. 18(2):110-21.

Repke KRH. 1988. The role of the na+/K+ pump in normal and cancer cell proliferation. In: Biomembranes: Basic and medical research. Benga G, Tager JM, editors. Berlin, Heidelberg: Springer Berlin Heidelberg. 160 p.

Resnick MJ, Koyama T, Fan K, Albertsen PC, Goodman M, Hamilton AS, Hoffman RM, Potosky AL, Stanford JL, Stroup AM, et al. 2013. Long-term

functional outcomes after treatment for localized prostate cancer. N Engl J Med. 368(5):436-45.

Rider JR, Wilson KM, Sinnott JA, Kelly RS, Mucci LA, Giovannucci EL. 2016. Ejaculation frequency and risk of prostate cancer: Updated results with an additional decade of follow-up. Eur Urol. 70(6):974-82.

Rifka SM, Pita JC,Jr, Loriaux DL. 1976. Mechanism of interaction of digitalis with estradiol binding sites in rat uteri. Endocrinology. 99(4):1091-6.

Rifka SM, Pita JC, Vigersky RA, Wilson YA, Loriaux DL. 1978. Interaction of digitalis and spironolactone with human sex steroid receptors. J Clin Endocrinol Metab. 46(2):338-44.

Rosenberg MT, Froehner M, Albala D, Miner MM. 2010. Biology and natural history of prostate cancer and the role of chemoprevention. Int J Clin Pract. 64(13):1746-53.

Rowles JL, Ranard KM, Smith JW, An R, Erdman JW. 2017. Increased dietary and circulating lycopene are associated with reduced prostate cancer risk: A systematic review and meta-analysis. Prostate Cancer and Prostatic Diseases. .

Ruskoaho H, Hakkola J, Huupponen R, Kantele A, Korpi E, Moilanen E, Piepponen P, Savontaus E, Tenhunen O, Vähäkangas K. 2014. Lääketieteellinen farmakologia ja toksikologia. Kustannus Oy Duodecim.

Ryan CJ, Smith MR, Fizazi K, Saad F, Mulders PF, Sternberg CN, Miller K, Logothetis CJ, Shore ND, Small EJ, et al. 2015. Abiraterone acetate plus prednisone versus placebo plus prednisone in chemotherapy-naive men with metastatic castration-resistant prostate cancer (COU-AA-302): Final overall survival analysis of a randomised, double-blind, placebo-controlled phase 3 study. Lancet Oncol. 16(2):152-60.

Saad F, Gleason DM, Murray R, Tchekmedyian S, Venner P, Lacombe L, Chin JL, Vinholes JJ, Goas JA, Chen B, et al. 2002. A randomized, placebo-controlled trial of zoledronic acid in patients with hormone-refractory metastatic prostate carcinoma. J Natl Cancer Inst. 94(19):1458-68.

Salas M, Hofman A, Stricker BH. 1999. Confounding by indication: An example of variation in the use of epidemiologic terminology. Am J Epidemiol. 149(11):981-3.

Salminen JK, Tammela TLJ, Auvinen A, Murtola TJ. 2016. Antiepileptic drugs with histone deacetylase inhibition activity and prostate cancer risk: A population-based case–control study. Cancer Causes & Control. 27(5):637-45.

Samson DJ, Seidenfeld J, Schmitt B, Hasselblad V, Albertsen PC, Bennett CL, Wilt TJ, Aronson N. 2002. Systematic review and meta-analysis of monotherapy compared with combined androgen blockade for patients with advanced prostate carcinoma. Cancer. 95(2):361-76.

Sayehmiri K, Azami M, Mohammadi Y, Soleymani A, Tardeh Z. 2018. The association between selenium and prostate cancer: A systematic review and meta-analysis. Asian Pac J Cancer Prev. 19(6):1431-7.

Scher HI, Fizazi K, Saad F, Taplin ME, Sternberg CN, Miller K, de Wit R, Mulders P, Chi KN, Shore ND, et al. 2012. Increased survival with enzalutamide in prostate cancer after chemotherapy. N Engl J Med. 367(13):1187-97.

Schröder FH, Hugosson J, Roobol MJ, Tammela TLJ, Zappa M, Nelen V, Kwiatkowski M, Lujan M, Määttänen L, Lilja H, et al. 2014. The european randomized study of screening for prostate cancer – prostate cancer mortality at 13 years of follow-up. Lancet. 384(9959):2027-35.

Schröder FH, Roobol MJ. 2010. ERSPC and PLCO prostate cancer screening studies: What are the differences? Eur Urol. 58(1):46-52.

Schumacher FR, Al Olama AA, Berndt SI, Benlloch S, Ahmed M, Saunders EJ, Dadaev T, Leongamornlert D, Anokian E, Cieza-Borrella C, et al. 2018. Association analyses of more than 140,000 men identify 63 new prostate cancer susceptibility loci. Nat Genet. 50(7):928-36.

Schwingshackl L, Hoffmann G. 2014. Adherence to mediterranean diet and risk of cancer: A systematic review and meta-analysis of observational studies. International Journal of Cancer. 135(8):1884-97.

Scott E, Adolfsson J, Aly M, Nordstrom T, Wiklund P, Brandberg Y, Thompson J, Wiklund F, Lindberg J, Clements M, et al. 2017. Prostate cancer screening in men aged 50 to 69 years (STHLM3): A prospective population-based diagnostic study. gronberg H, adolfsson J, aly M, nordstrom T, wiklund P, brandberg Y, thompson J, wiklund F, lindberg J, clements M, egevad L, eklund M.lancet oncol. 2015 dec;16(16):1667-76. [epub 2015 nov 10]. doi: 10.1016/S1470-2045(15)00361-7. Urol Oncol. 35(3):120.

Seidenfeld J, Samson DJ, Hasselblad V, Aronson N, Albertsen PC, Bennett CL, Wilt TJ. 2000. Single-therapy androgen suppression in men with advanced prostate cancer: A systematic review and meta-analysis. Ann Intern Med. 132(7):566-77.

Semenza GL. 2003. Targeting HIF-1 for cancer therapy. Nature Reviews Cancer. 3:721.

Severi G, Morris HA, MacInnis RJ, English DR, Tilley W, Hopper JL, Boyle P, Giles GG. 2006. Circulating steroid hormones and the risk of prostate cancer. Cancer Epidemiology and Prevention Biomarkers. 15(1):86-91.

Shang Z, Wang X, Yan H, Cui B, Wang Q, Wu J, Cui X, Li J, Ou T, Yang K. 2018. Intake of non-steroidal anti-inflammatory drugs and the risk of prostate cancer: A meta-analysis. Front Oncol. 8:437.

Sharma S, Symanowski J, Wong B, Dino P, Manno P, Vogelzang N. 2008. A phase II clinical trial of oral valproic acid in patients with castration-resistant prostate cancers using an intensive biomarker sampling strategy. Translational Oncology. 1(3):141-7.

Shibata M, Kavanaugh C, Shibata E, Abe H, Nguyen P, Otsuki Y, Trepel JB, Green JE. 2003. Comparative effects of lovastatin on mammary and prostate oncogenesis in transgenic mouse models. Carcin. 24(3):453-9.

Siegel RL, Miller KD, Jemal A. 2016. Cancer statistics, 2016. CA: A Cancer Journal for Clinicians. 66(1):7-30.

Siltari A, Murtola TJ, Talala K, Taari K, Tammela TLJ, Auvinen A. 2018. Antihypertensive drugs and prostate cancer risk in a finnish population-based cohort. Scand J Urol. 52(5-6):321-7.

Sinclair CS, Berry R, Schaid D, Thibodeau SN, Couch FJ. 2000. BRCA1 and BRCA2 have a limited role in familial prostate cancer. Cancer Res. 60(5):1371-5.

Su VY, Hu YW, Chou KT, Ou SM, Lee YC, Lin EY, Chen TJ, Tzeng CH, Liu CJ. 2013. Amiodarone and the risk of cancer: A nationwide population-based study. Cancer. 119(9):1699-705.

Suissa S. 2008. Immortal time bias in pharmaco-epidemiology. Am J Epidemiol. 167(4):492-9.

Sylvester JE, Grimm PD, Wong J, Galbreath RW, Merrick G, Blasko JC. 2011. Fifteen-year biochemical relapse-free survival, cause-specific survival, and overall survival following I(125) prostate brachytherapy in clinically localized prostate cancer: Seattle experience. Int J Radiat Oncol Biol Phys. 81(2):376-81.

Taari K, Aaltomaa S, Nurmi M, Parpala T, Tammela T. 2013. Urologia. Kustannus Oy Duodecim.

Taira AV, Merrick GS, Galbreath RW, Wallner KE, Butler WM. 2010. Natural history of clinically staged low- and intermediate-risk prostate cancer treated with monotherapeutic permanent interstitial brachytherapy. Int J Radiat Oncol Biol Phys. 76(2):349-54.

Tannock IF, de Wit R, Berry WR, Horti J, Pluzanska A, Chi KN, Oudard S, Theodore C, James ND, Turesson I, et al. 2004. Docetaxel plus prednisone or mitoxantrone plus prednisone for advanced prostate cancer. N Engl J Med. 351(15):1502-12.

Thelen P, Wuttke W, Seidlová-Wuttke D. 2014. Phytoestrogens selective for the estrogen receptor beta exert anti-androgenic effects in castration resistant prostate cancer. J Steroid Biochem Mol Biol. 139:290-3.

Thompson D, Easton DF, Breast Cancer Linkage Consortium. 2002. Cancer incidence in BRCA1 mutation carriers. J Natl Cancer Inst. 94(18):1358-65.

Thompson IM, Goodman PJ, Tangen CM, Lucia MS, Miller GJ, Ford LG, Lieber MM, Cespedes RD, Atkins JN, Lippman SM, et al. 2003. The influence of finasteride on the development of prostate cancer. N Engl J Med. 349(3):215-24.

Tsodikov A, Gulati R, Heijnsdijk EAM, Pinsky PF, Moss SM, Qiu S, de Carvalho TM, Hugosson J, Berg CD, Auvinen A, et al. 2017. Reconciling the effects of screening on prostate cancer mortality in the ERSPC and PLCO TrialsEffects of screening on prostate cancer mortality in ERSPC and PLCO. Aim. 167(7):449-55.

Uemura H, Ishiguro H, Nakaigawa N, Nagashima Y, Miyoshi Y, Fujinami K, Sakaguchi A, Kubota Y. 2003. Angiotensin II receptor blocker shows antiproliferative activity in prostate cancer cells: A possibility of tyrosine kinase inhibitor of growth factor. Mol Cancer Ther. 2(11):1139-47.

Veitonmaki T, Murtola TJ, Maattanen L, Taari K, Stenman UH, Tammela TL, Auvinen A. 2014. Prostate cancer risk and nonsteroidal antiinflammatory drug use in the finnish prostate cancer screening trial. Br J Cancer. 111(7):1421-31.

Vickers AJ, Cronin AM, Roobol MJ, Savage CJ, Peltola M, Pettersson K, Scardino PT, Schroder FH, Lilja H. 2010. A four-kallikrein panel predicts prostate cancer in men with recent screening: Data from the european randomized study of screening for prostate cancer, rotterdam. Clin Cancer Res. 16(12):3232-9.

Vickers AJ, Ulmert D, Sjoberg DD, Bennette CJ, Björk T, Gerdtsson A, Manjer J, Nilsson PM, Dahlin A, Bjartell A, et al. 2013. Strategy for detection of prostate cancer based on relation between prostate specific antigen at age 40-55 and long term risk of metastasis: Case-control study. Bmj. 346.

Vidal AC, Howard LE, Moreira DM, Castro-Santamaria R, Andriole GL, Freedland SJ. 2015. Aspirin, NSAIDs, and risk of prostate cancer: Results from the REDUCE study. Clin Cancer Res. 21(4):756-62.

Wacholder S, Hartge P, Lubin JH, Dosemeci M. 1995. Non-differential misclassification and bias towards the null: A clarification. Occup Environ Med. 52(8):557-8.

Wang Y, Liu X, Yan P, Tang J, Chen T, Sun Y, Zhou W, Bi Y, Zhang ZJ. 2019. Effect of metformin on the risk of prostate cancer in patients with type 2 diabetes by considering different confounding factors: A meta-analysis of observational studies. Eur J Cancer Prev. .

Wang Z, Wang Y, Zhang J, Hu Q, Zhi F, Zhang S, Mao D, Zhang Y, Liang H. 2017. Significance of the TMPRSS2:ERG gene fusion in prostate cancer. Mol Med Rep. 16(4):5450-8.

Weberpals J, Jansen L, Carr PR, Hoffmeister M, Brenner H. 2016. Beta blockers and cancer prognosis - the role of immortal time bias: A systematic review and meta-analysis. Cancer Treat Rev. 47:1-11.

Wettermark B, Zoëga H, Furu K, Korhonen M, Hallas J, Nørgaard M, Almarsdottir AB, Andersen M, Andersson Sundell K, Bergman U, et al. 2013. The nordic prescription databases as a resource for pharmacoepidemiological research—a literature review. Pharmacoepidemiol Drug Saf. 22(7):691-9.

ATC/DDD Index [Internet]. Available from: https://www.whocc.no/atc_ddd_index/ .

Wilt TJ, Brawer MK, Jones KM, Barry MJ, Aronson WJ, Fox S, Gingrich JR, Wei JT, Gilhooly P, Grob BM, et al. 2012. Radical prostatectomy versus observation for localized prostate cancer. N Engl J Med. 367(3):203-13.

Wilt TJ, Jones KM, Barry MJ, Andriole GL, Culkin D, Wheeler T, Aronson WJ, Brawer MK. 2017. Follow-up of prostatectomy versus observation for early prostate cancer. N Engl J Med. 377(2):132-42.

Wolf AM, Wender RC, Etzioni RB, Thompson IM, D'Amico AV, Volk RJ, Brooks DD, Dash C, Guessous I, Andrews K, et al. 2010. American cancer society guideline for the early detection of prostate cancer: Update 2010. CA Cancer J Clin. 60(2):70-98.

Wright JL, Hansten PD, Stanford JL. 2014. Is digoxin use for cardiovascular disease associated with risk of prostate cancer? Prostate. 74(1):97-102.

Xia Q, Sung J, Chowdhury W, Chen CL, Hoti N, Shabbeer S, Carducci M, Rodriguez R. 2006. Chronic administration of valproic acid inhibits prostate cancer cell growth in vitro and in vivo. Cancer Res. 66(14):7237-44.

Xie SH, Jernberg T, Mattsson F, Lagergren J. 2017. Digitalis use and risk of gastrointestinal cancers: A nationwide population-based cohort study. Oncotarget. 8(21):34727-35.

Zakikhani M, Dowling RJ, Sonenberg N, Pollak MN. 2008. The effects of adiponectin and metformin on prostate and colon neoplasia involve activation of AMP-activated protein kinase. Cancer Prev Res (Phila). 1(5):369-75.

Zamboglou N, Tselis N, Baltas D, Buhleier T, Martin T, Milickovic N, Papaioannou S, Ackermann H, Tunn UW. 2013. High-dose-rate interstitial brachytherapy as monotherapy for clinically localized prostate cancer: Treatment evolution and mature results. International Journal of Radiation Oncology • Biology • Physics. 85(3):672-8.

Zhang H, Qian DZ, Tan YS, Lee K, Gao P, Ren YR, Rey S, Hammers H, Chang D, Pili R, et al. 2008. Digoxin and other cardiac glycosides inhibit HIF-1α synthesis and block tumor growth. Proc Natl Acad Sci U S A. 105(50):19579-86.

Zhang Q, Feng H, Qluwakemi B, Wang J, Yao S, Cheng G, Xu H, Qiu H, Zhu L, Yuan M. 2017. Phytoestrogens and risk of prostate cancer: An updated meta-analysis of epidemiologic studies. Int J Food Sci Nutr. 68(1):28-42.

Zhong S, Yu D, Zhang X, Chen X, Yang S, Tang J, Zhao J, Wang S. 2016. Beta-blocker use and mortality in cancer patients: Systematic review and meta-analysis of observational studies. Eur J Cancer Prev. 25(5):440-8.

Zu K, Giovannucci E. 2009. Smoking and aggressive prostate cancer: A review of the epidemiologic evidence. Cancer Causes & Control. 20(10):1799-810.

PUBLICATION I

Sotalol, but not digoxin is associated with decreased prostate cancer risk: A population-based case-control study

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Sotalol, but not digoxin is associated with decreased prostate cancer risk: A population-based case–control study

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Antiarrhythmic drug digoxin has been reported to have apoptosis-inducing and cytotoxic effects on prostate cancer cells. We evaluated the association between antiarrhythmic drug use and prostate cancer risk in a population-based case–control study. The study included all new prostate cancer cases diagnosed in Finland during 1995-2002 and matched controls (24,657 case–control pairs) obtained from the Finnish Cancer Registry and the Population Register Center, respectively. Information on antiarrhythmic drug purchases was obtained from national prescription database. Multivariable-adjusted conditional logistic regression model was used for data analysis. Compared to never-users of antiarrhythmic drugs, we found no significant association between digoxin use and prostate cancer risk overall [odds ratio (OR) 0.95, 95% confidence interval (CI): 0.89–1.01] or for advanced prostate cancer risk (OR: 0.90, 95% CI: 0.77–1.05). The result was similar also for other antiarrhythmic drugs, with the exception of sotalol, users of which had decreased risk of advanced prostate cancer (OR: 0.73, 95% CI: 0.56–0.96). Also the overall prostate cancer risk decreased by duration of sotalol use (p for trend 0.038). We show that digoxin or other common antiarrhythmic drugs generally do not associate with prostate cancer risk at population level during maximum follow-up of eight years. However, we cannot rule out longer term protective effects of digoxin. K^+-channel blocker sotalol shows some promise as prostate cancer preventing agent. However, findings need to be confirmed in further studies.

Prostate cancer is the most common malignancy among men in most countries.[1] Despite being a major public health problem, its etiology is still not well-known. A deeper knowledge of the risk factors is needed for prevention and better treatment of the condition.

Digoxin, a commonly used antiarrhythmic agent, inhibits prostate cancer cell growth by increasing apoptosis.[2,3] The mechanism of action for cardiac glycosides such as digoxin involves inhibition of the plasma membrane Na+/K+-ATPase, leading to changes in intracellular K+- and Ca2+-concentrations. The apoptosis-inducing effect of digoxin has been proposed to be caused by increased Ca2+-uptake in prostate cancer cells,[2,3] leading to changes in activity of cyclin-dependent kinase Cdk5, p35 cleavage and p25 formation.[4] Cardiac glycosides also decrease prostate specific antigen (PSA) secretion in prostate cancer cells.[5] In a mouse model digoxin treatment caused decreased blood vessel density and inhibition of HIF-1α expression in castration-resistant xenograft tumors, but no reduction in tumor volume.[6]

On the other hand, digoxin possesses estrogen-mimicking effects, and its use is associated with an increased incidence of breast and uterine cancer.[7] This would provide another plausible mechanism for prostate cancer inhibiting effects.

Recently, a novel two-stage study confirmed, at the first stage, the cytotoxic effects of digoxin against prostate cancer cells in an *in vitro* cytotoxicity screen. [8] At the second stage, the study also reported decreased prostate cancer incidence among men who had used digoxin regularly for over ten years within a cohort of 47,884 men.[8] Furthermore, coherent

Key words: antiarrhythmic drugs, digoxin, incidence, prostate cancer, sotalol

Abbreviations: BPH: benign prostatic hyperplasia; BMI: body mass index; CI: confidence interval; DDD: defined daily dose; HIF: hypoxia-inducible factor; NSAID: non-steroidal anti-inflammatory drug; OR: odds ratio; PSA: prostate-specific antigen; SII: Social Insurance Institution of Finland

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What's new?

The antiarrhythmic drug digoxin triggers apoptosis in prostate cancer cells, and recent research suggests that the drug may even reduce prostate cancer risk. In the present population-based case-control study, which included data on more than 25,000 Finnish men, digoxin and other antiarrhythmic drugs, with the exception of sotalol, were found to have no impact on prostate cancer risk. By contrast, sotalol, which possesses both beta-blocker and K+-channel inhibitor activity, was inversely associated with overall risk and risk of advanced prostate cancer. If validated, sotalol may prove to be of greater relevance to prostate cancer prevention than digoxin.

results were reported in a recent epidemiological case-control study. The study showed the prostate cancer risk was decreased in digoxin users especially among men with 3 or more PSA-tests during the past five years.[9]

We evaluated whether the use of digoxin or, for comparison, other antiarrhythmic agents is related to overall or advanced prostate cancer risk at population level.

Material and Methods

Study population

We used a nationwide case-control study population including all newly diagnosed prostate cancer cases in Finland during 1995–2002. The study population has been extensively described previously.[10–13]

In brief, all new prostate cancer cases in Finland between 1995 and 2002 (25,029 men) were obtained from the Finnish Cancer Registry. The nationwide registry covers over 99% of all prostate cancer patients in Finland.[14] The registry data includes information on primary site of cancer, histology, date and method of diagnosis. Tumor stage was available for 13,616 cases (55% of all cases). Of these, 73% were localized. The registry had no information on Gleason score or PSA values or screening activity prior to the diagnosis.

Practically all the cases were histologically confirmed (99.3%). In a small portion the diagnosis was based on clinical (0.4%), radiological (0.3%), other specific laboratory findings (0.02%). 185 cases were excluded for unknown method of diagnosis (0.7%). In addition, 66 duplicate cases were excluded.

For each case, the Population Registry Center of Finland randomly selected a control from among the men who were of the same age (±1 year), living in the same area and were free of prostate cancer at the time of the cases' diagnosis. We used incidence density sampling for control selection, and thus 963 men were considered twice in the analysis; first as a control and then as a case in another case-control pair after being diagnosed with prostate cancer at a later time. Matched controls could not be found for 121 cases in the oldest age group. In the end, a total of 24,657 individually matched case-control pairs were included in the analysis.

The study was approved by the ethics committee of the Pirkanmaa health care district, Finland (ETL R03290).

Information on medication use

Information on reimbursed physician-prescribed medication purchases during 1995–2002 was obtained from the comprehensive nationwide prescription database of the Social Insurance Institution (SII) of Finland. The SII is a governmental agency financed through tax revenues, providing reimbursements for the cost of medicines prescribed by a physician with the exception of hospital inpatients.[15] The reimbursement is available for all Finnish residents, for each purchase of a SII approved reimbursable drug and covers 35–100% of the costs depending on the severity of the disease. For example, the level of the reimbursement is 100% to the prostate cancer and antidiabetic drugs whereas to the antiarrhythmic and antihypertensive drugs it is 65%. The reimbursements are being used by approximately 95% of Finnish citizens. In practice, the reimbursement is most often received as price discount of purchased medication at pharmacy.

We included all antiarrhythmic agents being used within our study population during 1995–2002. Beta-blockers, which are used both as antihypertensive and as antiarrhythmic drugs, have been analyzed in an earlier study along with antihypertensive medication.[11] In this study was we analyzed sotalol, which is a beta-blocker but mainly used as an antiarrhythmic agent, by itself and also along with other beta-blockers.

The drugs included in this analysis were amiodarone, digoxin, disopyramide, etilefrine, flecainide, quinidine, mexiletine, propafenone, sotalol, tocainide, pindolol, propranolol, timolol, acebutolol, atenolol, betaxolol, bisoprolol, metoprolol, celiprolol, carvedilol, labetalol, oxprenolol and alprenolol. Of these, quinidine, disopyramide, mexiletine, tocainide, propafenone and flecainide function as Na+-channel inhibitors. Amiodarone and sotalol are K+-channel inhibitors. Sotalol also has beta-blocker function. Etilefrine is a sympathomimetic agent that is suggested to stimulate both alpha- and beta-receptors. Digoxin's mechanism of action is not completely understood, but it involves an effect on Na+/K+ ATPase pump. Pindolol, propranolol, timolol, carvedilol, labetalol, oxprenolol, alprenolol and sotalol are non-selective beta-blockers whereas acebutolol, atenolol, betaxolol, bisoprolol, metoprolol and celiprolol function as β1-selective beta-blockers. Furthermore, beta-blockers can be divided into hydrophilic (atenolol, celiprolol and sotalol) and hydrophobic drugs.

Statistical analysis

Medication use was followed from January 1st, 1995 up to the month of diagnosis of the prostate cancer cases. For the

Table 1. Prevalence of Medication Use Among the Study Population of 24,657 Diagnosed Prostate Cancer Cases in Finland in 1995–2002 and Their Individually Matched Controls

	Cases		Controls	
	No.	%	No.	%
Total	24,657	50	24,657	50
Digoxin use	2,616	10.6	2,550	10.3
Any antiarrhythmic drug use	3,408	13.8	3,316	13.4
Non-steroidal anti-inflammatory drugs	13,265	53.8	11,475	46.5
Benign prostatic hyperplasia medication[1]	4,603	18.7	3,086	12.5
Anti-diabetic medication[2]	2,209	9.0	2,391	9.7
Cholesterol-lowering medication[3]	2621	10.6	2,439	9.9
Antihypertensive medication[4]	12,719	51.6	11,749	47.6

[1]Includes finasteride and alpha-blockers tamsulosin and alfuzosin.
[2]Includes oral antidiabetic medication and insulin.
[3]includes statins, fibric acid derivatives, bile acid binding resins and acipimox.
[4]includes diuretics, beta-blockers, calcium-channel blockers, angiotensin-converting enzyme inhibitors and angiotensin receptor blockers.

controls, medication use was followed until the date of diagnosis of the corresponding matched case, ensuring equal available exposure time for the cases and the controls.

The amount of antiarrhythmic medication use was standardized across the drug groups by dividing the purchased mg amount of a drug with a quantity corresponding one Defined Daily Dose (DDD) recommended by the WHO.[16] Total number of DDDs for each individual drug was combined for a total sum of DDDs of all antiarrhythmic drugs used by each person.

Propensity for antiarrhythmic drug usage as function of age and usage of other types of drugs was estimated using a logistic regression method with antiarrhythmic drug usage as the dependent variable and age, use of 5α-reductase inhibitors, alpha-blockers, anti-diabetic drugs, cholesterol-lowering drugs and antihypertensive drugs as independent variables. The propensity score for each was added together for total propensity score. The analysis was repeated by quartiles of the total propensity score to estimate the drugs effect within subpopulations that are comparable in their likelihood to use antiarrhythmic drugs.

Non-users of antiarrhythmic drugs were the reference group in all analyses. Analyses of individual drugs were restricted to users of the drug under analyses. We used conditional logistic regression to calculate ORs and 95% CIs for overall prostate cancer risk and risk of advanced prostate cancer among the medication users. The analysis was adjusted for age alone (age-adjusted model) or additionally for use of the above mentioned drug groups (multivariable-adjusted model).

Each drug was analyzed separately and in combination with other antiarrhythmic drugs. We compared prostate cancer risk by ever-use of the drugs, and also in analysis stratified in quartiles of the amount, duration and intensity of medication use. We analyzed trends in prostate cancer risk by cumulative medication use by adding total DDDs or years of usage into the logistic regression model as a continuous variable.

In sensitivity analyses we aimed to estimate the effect of previous PSA-testing by stratifying the analysis by simultaneous usage of drugs used in management of benign prostatic hyperplasia (BPH); 5α-reductase inhibitors and alpha-blockers. Furthermore, we stratified the analysis both to the users and non-users of statins, NSAIDs, antidiabetic drugs and antihypertensive drugs. p for interaction was calculated for all drugs above.

The data was analyzed using Stata 8.2 software (College Station, Texas).

Results

Population characteristics

Overall, the prevalence of digoxin use was similar between the cases and controls; 10.6% for the cases (2,616 men) and 10.3% for the controls (2,550 men) (Table 1). The overall prevalence of antiarrhythmic drug use was 13.6%, with no difference between cases and controls. Of the other drug groups, prevalence of use for non-steroidal anti-inflammatory drugs (NSAIDs), BPH medication, cholesterol-lowering medication and antihypertensive drugs was higher among the cases, whereas the prevalence of antidiabetic medication use was lower (Table 1).

Digoxin use and prostate cancer

Digoxin use was not significantly associated with overall prostate cancer risk either in the age-adjusted analysis (OR: 1.03, 95% CI: 0.97–1.09) or the multivariable-adjusted analysis (OR: 0.96, 95% CI: 0.90–1.02). (Table 2).

The risk of advanced prostate cancer in digoxin users was lower than the overall prostate cancer risk (multivariable-adjusted OR: 0.89, 95% CI: 0.76–1.05), but the difference compared to the non-users remained non-significant (Table 2).

There was no association between digoxin use and prostate cancer risk, either overall or advanced, within any quartile of digoxin usage (Table 3). The multivariable-adjusted trend analysis in prostate cancer risk by cumulative amount or duration of digoxin use likewise showed no associations with overall or advanced prostate cancer.

Other antiarrhythmic drugs and prostate cancer

Usage of any antiarrhythmic drug use was not associated with the risk of either overall (OR: 0.96, 95% CI: 0.91–1.01) or advanced prostate cancer risk (OR: 0.90, 95% CI: 0.77–1.04) (Table 2). The difference between users and non-users of antiarrhythmic drugs remained non-significant in each quartile of total DDD amount of use, although there was a borderline significant decreasing trend in overall prostate cancer risk by duration of usage (p for trend = 0.058) (Table 3).

Table 2. Age-adjusted and Multivariable-adjusted Odds Ratios and 95% Confidence Intervals for Any Prostate Cancer and Advanced Prostate Cancer in Users of Antiarrhythmic Drugs Compared With Nonusers Among 24,657 Finnish Prostate Cancer Cases and Matched Controls During 1995–2002

	Overall prostate cancer			Advanced disease[1]		
Drug type	N of cases	OR (95% CI) age-adjusted	OR (95% CI) multivar.-adjusted[2]	N of cases	OR (95% CI) age-adjusted	OR (95% CI) multivar.-adjusted[2]
Any antiarrhythmic drug	3,408	1.03 (0.98–1.09)	0.96 (0.91–1.01)	465	0.92 (0.80–1.05)	0.90 (0.77–1.04)
Digoxin	2,616	1.03 (0.97–1.09)	0.96 (0.90–1.02)	375	0.92 (0.79–1.07)	0.89 (0.76–1.05)
Na^+-channel blockers	378	1.06 (0.92–1.23)	0.98 (0.84–1.13)	39	0.78 (0.51–1.19)	0.76 (0.50–1.18)
Quinidine	207	1.04 (0.85–1.26)	0.94 (0.77–1.15)	22	0.73 (0.41–1.27)	0.73 (0.41–1.31)
Disopyramide	93	1.11 (0.82–1.49)	1.04 (0.77–1.40)	14	1.56 (0.68–3.61)	1.73 (0.74–4.05)
Mexiletine	44	1.05 (0.69–1.60)	0.93 (0.61–1.43)	5	0.71 (0.23–2.25)	0.61 (0.19–1.97)
Propafenone	47	1.42 (0.91-2.22)	1.21 (0.77–1.91)	4	0.80 (0.21–2.98)	0.55 (0.15–2.06)
Flecainide	84	1.22 (0.89–1.68)	1.05 (0.76–1.45)	4	0.44 (0.14–1.44)	0.35 (0.10–1.15)
K^+-channel blockers	858	1.01 (0.92–1.12)	0.94 (0.85–1.03)	103	0.78 (0.60–1.01)	0.78 (0.60–1.02)
Amiodarone	99	1.05(0.79–1.40)	0.99 (0.74–1.32)	11	1.57 (0.61–4.05)	1.74 (0.66–4.57)
Sotalol	823	1.02 (0.93–1.13)	0.94 (0.85–1.04)	98	0.73 (0.56–0.96)	0.73 (0.56–0.96)
Beta-blockers						
β1-selective[3]	6,516	1.06 (1.02–1.10)	1.00 (0.96–1.05)	844	0.99 (0.64–1.79)	1.00 (0.88–1.12)
Non-selective[4]	2,370	1.04 (0.98–1.10)	0.99 (0.93–1.06)	296	0.85 (0.72–1.00)	0.85 (0.72–1.00)
w/o sotalol	1,646	1.06 (0.99–1.14)	1.03 (0.96–1.11)	211	0.95 (0.78–1.16)	0.95 (0.78–1.16)
Hydrophilic[5]	2,284	1.04 (0.98–1.11)	1.00 (0.93–1.06)	272	0.81 (0.68–0.96)	0.80 (0.68–0.96)
w/o sotalol	1,537	1.04 (0.97–1.12)	1.01 (0.93–1.08)	183	0.86 (0.70–1.05)	0.84 (0.68–1.04)
Other:						
Etilefrine	133	0.87 (0.69–1.10)	0.80 (0.63–1.01)	13	0.72 (0.35–1.48)	0.66 (0.32–1.37)

[1]Includes all stage T3 and T4, N+ and M+ tumors.
[2]Calculated with conditional logistic regression adjusted for age and use of antihypertensive drugs, cholesterol-lowering drugs, antidiabetic drugs, non-steroidal anti-inflammatory drugs, 5α-reductase inhibitors and alpha-blockers.
[3]Includes acebutolol, atenolol, betaxolol, bisoprolol, metoprolol and celiprolol.
[4]Includes sotalol, pindolol, propranolol, timolol, carvedilol, labetalol, oxprenolol and alprenolol.
[5]Includes sotalol, atenolol and celiprolol.

When analyzed separately, use of Na^+-channel blockers was not associated with prostate cancer risk (Table 2). No individual Na^+-channel blocker had an effect on overall or advanced prostate cancer risk, either. The amount or duration of Na^+-channel blockers use did not affect the risk in the analysis stratified by quartiles (Table 3) nor in the trend analysis.

The K^+-channel blockers amiodarone and sotalol as a group were not associated with either overall or advanced prostate cancer risk; multivariable-adjusted OR: 0.94, 95% CI: 0.85–1.03 and OR: 0.78, 95% CI: 0.60–1.02, respectively (Table 2). However, use of sotalol was associated with a decreased risk of advanced prostate cancer; OR: 0.73, 95% CI: 0.56–0.96 (Table 2). Also overall prostate cancer risk was lower among men who had used sotalol for five years or longer; OR: 0.68, 95% CI: 0.55–0.85 (Table 3). The association with advanced prostate cancer risk was not dose-dependent, but we observed an inverse association between overall prostate cancer risk and years of sotalol usage (p for trend = 0.038) (Table 3).

Sensitivity analyses

A sensitivity analysis stratified by quartiles of propensity score confirmed the lack of association between prostate cancer and use of digoxin or other antiarrhythmic drugs, confirming that the association is not modified by the likelihood of being a user of this drug group (Table 4). The risk estimates of advanced prostate cancer among sotalol users were uniformly lowered, but statistically non-significant.

An analysis stratified by simultaneous use of BPH drugs (5α-reductase inhibitors and/or alpha-blockers) showed no clear effect modification, either (Table 4). The risk of advanced prostate cancer was decreased among sotalol users regardless of BPH-medication usage (OR: 0.21, 95% CI: 0.04–

Table 3. Multivariable-adjusted Odds Ratios (With 95% Confidence Intervals) of Prostate Cancer Overall and Advanced Prostate Cancer by the Amount and Duration of Antiarrhythmic Drug use Among 24,657 Finnish Prostate Cancer Cases and Matched Controls During 1995–2002

	Any antiarrhythmic drug		Na+-channel blockers		K+-channel blockers		Digoxin		Sotalol	
	Overall PCa	Advanced PCa[1]	Overall PCa	Advanced PCa	Overall PCa	Advanced PCa	Overall PCa	Advanced PCa	Overall PCa	Advanced PCa
Cumulative total amount of medication use[3]	OR (95% CI) [2]	OR (95% CI)	OR (95% CI)	OR (95% CI)	OR (95% CI)	OR (95% CI)	OR (95% CI)	OR (95% CI)	OR (95% CI)	OR (95% CI)
1st quartile	0.98 (0.88–1.08)	0.99 (0.76–1.29)	0.89 (0.67–1.18)	0.51 (0.21–1.22)	1.01 (0.84–1.23)	0.66 (0.38–1.17)	1.00 (0.89–1.13)	0.90 (0.67–1.21)	1.02 (0.84–1.24)	0.72 (0.41–1.27)
2nd quartile	0.93 (0.84–1.03)	0.70 (0.54–0.92)	1.17 (0.88–1.54)	0.85 (0.36–2.00)	0.88 (0.73–1.06)	0.70 (0.44–1.14)	0.90 (0.80–1.00)	0.73 (0.56–0.97)	0.93 (0.76–1.15)	0.56 (0.33–0.96)
3rd quartile	0.99 (0.89–1.09)	0.97 (0.75–1.27)	0.94 (0.71–1.26)	1.12 (0.52–2.40)	1.03 (0.85–1.26)	0.79 (0.47–1.34)	1.02 (0.91–1.14)	0.94 (0.70–1.27)	0.97 (0.80–1.18)	0.73 (0.44–1.21)
4th quartile	0.93 (0.84–1.03)	0.96 (0.72–1.29)	0.97 (0.73–1.28)	0.65 (0.28–1.49)	0.85 (0.70–1.04)	1.01 (0.59–1.74)	0.94 (0.83–1.05)	1.10 (0.78–1.55)	0.83 (0.67–1.01)	0.99 (0.56–1.73)
OR (95% CI)trend *p* for trend	1.00 (1.00–1.00) 0.179	1.00 (1.00–1.00) 0.323	1.00 (1.00–1.00) 0.995	1.00 (1.00–1.00) 0.326	1.00 (1.00–1.00) 0.125	1.00 (1.00–1.00) 0.542	1.00 (1.00–1.00) 0.438	1.00 (1.00–1.00) 0.760	1.00 (1.00–1.00) 0.083	1.00 (1.00–1.00) 0.469
Duration of medication use										
1 year	0.97 (0.88–1.07)	0.90 (0.70–1.16)	1.03 (0.80–1.31)	0.50 (0.25–1.00)	0.97 (0.82–1.14)	0.75 (0.47–1.21)	0.99 (0.88–1.10)	0.88 (0.68–1.15)	1.00 (0.85–1.19)	0.70 (0.43–1.15)
2 years	1.00 (0.89–1.12)	0.88 (0.65–1.18)	0.97 (0.71–1.33)	1.32 (0.45–3.86)	0.93 (0.75–1.15)	0.56 (0.33–0.95)	0.99 (0.87–1.13)	0.91 (0.66–1.27)	0.90 (0.72–1.13)	0.54 (0.31–0.92)
3–4 years	0.97 (0.88–1.07)	0.90 (0.70–1.16)	0.97 (0.74–1.27)	0.92 (0.46–1.87)	1.12 (0.93–1.36)	1.31 (0.78–2.21)	0.93 (0.84–1.04)	0.86 (0.64–1.14)	1.15 (0.94–1.40)	1.05 (0.62–1.78)
5 years or longer	0.90 (0.81–1.00)	0.90 (0.66–1.22)	0.96 (0.70–1.32)	0.90 (0.29–2.86)	0.72 (0.59–0.90)	0.65 (0.35–1.21)	0.94 (0.84–1.07)	0.95 (0.66–1.35)	0.68 (0.55–0.85)	0.73 (0.39–1.36)
OR (95% CI)trend *p* for trend [4]	0.99 (0.97–1.00) 0.058	0.97 (0.93–1.01) 0.175	0.99 (0.95–1.03) 0.721	0.96 (0.84–1.10) 0.581	0.97 (0.95–1.00) 0.073	0.95 (0.88–1.03) 0.252	0.99 (0.97–1.01) 0.172	0.97 (0.93–1.02) 0.227	0.97 (0.94–1.00) 0.038	0.95 (0.87–1.03) 0.189
Intensity of medication use (DDDs/year)										
1st quartile	1.00 (0.91–1.09)	0.95 (0.72–1.21)	0.86 (0.65–1.14)	0.56 (0.24–1.29)	0.97 (0.82–1.14)	0.70 (0.43–1.13)	0.98 (0.89–1.08)	0.85 (0.67–1.09)	0.97 (0.81–1.14)	0.69 (0.43–1.12)
2nd quartile	0.89 (0.79–1.00)	0.82 (0.60–1.12)	1.18 (0.90–1.55)	1.03 (0.47–2.27)	0.99 (0.79–1.24)	0.71 (0.40–1.28)	0.90 (0.79–1.02)	0.72 (0.52–1.00)	1.14 (0.88–1.46)	0.68 (0.36–1.30)
3rd quartile	0.91 (0.83–1.01)	0.77 (0.59–1.01)	0.89 (0.67–1.19)	1.04 (0.45–2.40)	0.89 (0.73–1.08)	0.65 (0.38–1.11)	0.93 (0.82–1.06)	0.88 (0.62–1.26)	0.81 (0.67–0.99)	0.65 (0.38–1.09)
4th quartile	1.02 (0.92–1.12)	1.02 (0.77–1.34)	1.03 (0.78–1.37)	0.58 (0.26–1.31)	0.92 (0.76–1.12)	1.14 (0.68–1.92)	1.01 (0.90–1.14)	1.16 (0.85–1.57)	0.93 (0.76–1.14)	0.95 (0.55–1.65)
OR (95% CI)trend *p* for trend [4]	1.00 (1.00–1.00) 0.429	1.00 (1.00–1.00) 0.368	1.00 (1.00–1.00) 0.627	1.00 (1.00–1.00) 0.201	1.00 (1.00–1.00) 0.315	1.00 (1.00–1.00) 0.429	1.00 (1.00–1.00) 0.658	1.00 (1.00–1.00) 0.800	1.00 (1.00–1.00) 0.276	1.00 (1.00–1.00) 0.185

[1]Includes all stage T3 and T4, N+ and M+ tumors.
[2]Calculated with conditional logistic regression adjusted for age and use of antihypertensive drugs, cholesterol-lowering drugs, antidiabetic drugs, non-steroidal anti-inflammatory drugs, 5α-reductase inhibitors and alpha-blockers.
[3]Quartile cut-points: Any arrhythmic drugs 1: 6-150 DDD, 2: 155-445DDD, 3:450-950 DDD, 4: 955 DDD or more; Na^+-channel blockers 1: 5-77 DDD, 2: 78-350 DDD, 3: 351-840 DDD 4: 841 DDD or more; K^+-channel blockers 1: 15-100 DDD, 2: 101-400 DDD, 3: 401-900 DDD 4: 901 DDD or more; digoxin 1:5-145 DDD, 2: 150-390 DDD, 3: 400-800 DDD, 4: 810 DDD or more and sotalol 1: 15-100 DDD, 2: 105-395 DDD, 3: 400-900 DDD, 4: 915 DDD or more.
[4]Calculated by analyzing the cumulative medication use as a continuous variable.

Table 4. Risk of Prostate Cancer Overall and Advanced Prostate Cancer in Users of Antiarrhythmic Drugs, Digoxin and Sotalol Among 24,657 Finnish Prostate Cancer Cases and Matched Controls During 1995–2002. Analysis Stratified by Propensity score and baseline variables

	Any antiarrhythmic drug use		Digoxin users		Sotalol users	
	Overall PCa	Advanced PCa[1]	Overall PCa	Advanced PCa[1]	Overall PCa	Advanced PCa[1]
Propensity score	OR (95% CI)[2]	OR (95% CI)[2]	OR (95% CI)[2]	OR (95% CI)[2]	OR (95% CI)[2]	OR (95% CI)[2]
1st quartile	1.05 (0.72–1.54)	0.70 (0.19–2.52)	0.66 (0.37–1.16)	2.00 (0.18–22.06)	1.04 (0.60–1.80)	0.30 (0.06–1.44)
2nd quartile	0.94 (0.75–1.19)	0.57 (0.32–1.03)	0.86 (0.65–1.15)	0.38 (0.19–0.76)	0.89 (0.57–1.40)	0.34 (0.09–1.26)
3rd quartile	1.01 (0.87–1.18)	0.81 (0.50–1.29)	0.99 (0.83–1.17)	0.84 (0.49–1.44)	1.24 (0.95–1.61)	0.63 (0.28–1.41)
4th quartile	1.01 (0.88–1.16)	0.91 (0.64–1.29)	0.99 (0.86–1.14)	0.80 (0.55–1.16)	0.99 (0.77–1.26)	0.64 (0.34–1.23)
p for interaction	0.025	0.233	0.049	0.822	0.090	0.102
Baseline age						
Younger than 68	0.96 (0.88–1.06)	0.94 (0.71–1.24)	0.95 (0.85–1.07)	0.97 (0.69–1.36)	1.07 (0.93–1.25)	0.77 (0.51–1.17)
68 or older	0.95 (0.89–1.02)	0.88 (0.74–1.05)	0.96 (0.90–1.04)	0.88 (0.73–1.05)	0.84 (0.73–0.96)	0.69 (0.48–1.00)
p for interaction	0.075	0.554	0.196	0.696	0.083	0.105
BPH medication usage						
Users	0.83 (0.63–1.08)	1.18 (0.47–2.93)	0.94 (0.70–1.26)	0.98 (0.35–2.76)	0.75 (0.49–1.16)	0.21 (0.04–1.24)
Non-users	1.01 (0.95–1.08)	0.88 (0.74–1.04)	1.01 (0.94–1.09)	0.92 (0.77–1.11)	0.95 (0.84–1.08)	0.70 (0.50–0.96)
p for interaction	0.365	0.001	0.632	0.001	0.115	0.340
Statin usage						
Users	1.04 (0.68–1.54)	0.75 (0.18–3.04)	0.82 (0.52–1.30)	0.77 (0.16–3.85)	0.84 (0.48–1.47)	0.87 (0.16–4.80)
Non-users	0.94 (0.89–1.01)	0.87 (0.74–1.03)	0.97 (0.91–1.04)	0.89 (0.75–1.06)	0.86 (0.77–0.98)	0.61 (0.45–0.84)
p for interaction	0.845	0.035	0.194	0.002	0.708	0.823
NSAID usage						
Users	0.99 (0.89–1.09)	0.99 (0.75–1.30)	1.00 (0.89–1.11)	0.95 (0.70–1.29)	0.91 (0.77–1.07)	0.67 (0.42–1.08)
Non-users	0.96 (0.86–1.09)	0.78 (0.57–1.05)	0.98 (0.87–1.12)	0.71 (0.51–0.99)	0.97 (0.77–1.23)	0.81 (0.43–1.55)
p for interaction	0.194	0.926	0.277	0.670	0.383	0.519
Antidiabetic drug usage						
Users	0.96 (0.62–1.48)	0.55 (0.14–2.13)	0.91 (0.58–1.43)	0.38 (0.09–1.65)	1.20 (0.41–3.53)	1.48 (0.15–14.67)
Non-user	0.97 (0.91–1.03)	0.92 (0.78–1.10)	0.96 (0.89–1.03)	0.93 (0.77–1.13)	0.96 (0.86–1.08)	0.79 (0.57–1.10)
p for interaction	0.002	0.048	0.008	0.030	0.300	0.443
Antihypertensive drug usage						
Users	0.99 (0.91–1.08)	0.84 (0.67–1.05)	0.98 (0.89–1.07)	0.82 (0.64–1.05)	1.03 (0.88–1.20)	0.64 (0.42–0.97)
Non-users	0.90 (0.75–1.09)	0.58 (0.35–0.96)	0.82 (0.64–1.05)	0.44 (0.23–0.83)	0.88 (0.64–1.22)	0.33 (0.13–0.84)
p for interaction	0.550	0.242	0.450	0.440	0.809	0.518

[1]Includes all stage T3 and T4, N+ and M+ tumors.
[2]Calculated with conditional logistic regression adjusted for age and use of antihypertensive drugs, cholesterol-lowering drugs, antidiabetic drugs, non-steroidal anti-inflammatory drugs, 5α-reductase inhibitors and alpha-blockers.

1.24 and OR: 0.70, 95% CI: 0.50–0.96 among users and non-users of BPH medication, respectively. *p* for interaction 0.340) (Table 4). Neither simultaneous usage of statins, NSAIDs, antidiabetic drugs nor antihypertensive drugs had interaction with the risk of advanced prostate cancer among sotalol users (Table 4).

When we limited the analysis to include only cases diagnosed during 2000–2002 to see whether the association differs by the period of diagnosis, both digoxin use and antiarrhythmic drug use in general were associated with slight reduction in overall prostate cancer risk (OR: 0.89, 95% CI: 0.80–0.98 and OR: 0.91, 95% CI: 0.84–0.99, respectively), but no trend by the cumulative dose of medication use was observed for either. The risk of advanced prostate cancer among digoxin, sotalol and any antiarrhythmic drug users did not differ significantly from the non-users in this sensitivity analysis. We further evaluated impact of long-term exposure by limiting the exposure to usage during 1995–1997 in this same sensitivity analysis. The risk estimates for overall prostate cancer risk remained similar (OR: 0.88, 95% CI: 0.80–0.97 and OR: 0.93, 95% CI: 0.83–1.04 for antiarrhythmic drugs in general and for digoxin, respectively).

We compared prostate cancer risk between digoxin users and other antiarrhythmic drug users. No significant difference was found in overall prostate cancer risk (OR: 1.27, 95% CI: 0.94–1.70) or risk of advanced disease (OR: 1.66, 95% CI: 0.72–3.84).

Beta-blockers and prostate cancer risk

The usage of β1-selective beta-blockers did not affect to the risk of the prostate cancer; multivariable-adjusted OR: 1.00, 95% CI: 0.96–1.05 (Table 2). Neither use of non-selective nor hydrophilic beta-blockers had association with the overall prostate cancer risk (OR: 0.99, 95% CI: 0.93–1.06 and OR: 1.00, 95% CI: 0.93–1.06). Nevertheless, the risk of the advanced prostate cancer was slightly decreased among the users of the non-selective and hydrophilic beta-blockers (OR: 0.85, 95% CI: 0.72–1.00 and OR: 0.80, 95% CI: 0.68–0.96) (Table 2). Once the same analyze was performed without sotalol, the association with the advanced disease was no longer observed for non-selective beta-blockers (OR: 0.95, 95% CI: 0.78–1.16), but for hydrophilic beta-blockers a borderline significant risk decrease was observed even without sotalol (OR: 0.84, 95% CI: 0.68–1.04) (Table 2).

Discussion

Our results suggest that use of digoxin or other antiarrhythmic agents in general does not affect prostate cancer risk at population level with maximum exposure time of eight years. However, sotalol use was inversely associated both with advanced prostate cancer risk, and long-term users also had lower risk of overall prostate cancer. This drug deserves further study as prostate cancer preventive agent.

A previous study has reported promising results of digoxin's inhibitory effects on prostate cancer cell lines and also that regular long-term use of digoxin is related to a lower prostate cancer risk in the Health Professionaĺs Follow-up Study.[8] In the present study we found no evidence of lower overall or advanced prostate cancer risk among digoxin users at population-level. Furthermore, we found no dose-response in the risk by the cumulative amount or duration of digoxin use. The difference between our results and the earlier study is likely explained with our exposure time being eight years at maximum. In the Health Professional's Follow-up Study the decreased prostate cancer risk among digoxin users was driven by the very long-term users, *i.e.,* when the analysis was stratified by duration of digoxin use the risk decrease was observed only in men who had used the drug for 10 years or longer.[8] In concordance to our results, digoxin use for <10 years was not associated with prostate cancer risk in Health Professional's Follow-up Study, either.[8] This strongly suggests that digoxin's protective effects against prostate cancer likely require a considerably long induction period when the drug is being used at the clinical dose range. This lessens the enthusiasm to study digoxin's prostate cancer preventive effects in clinical trials. However, our study does not consider the possible influence of digoxin use to the progression of the cancer and further research of digoxin use and prostate cancer outcomes would be reasonable.

Our results differ from a recent case-control study which reported up to 66% lower prostate cancer risk in men using digoxin.[9] However, the exposure information in the previous study relied on survey information, thus possibly influenced by recall bias. In our study information on exposure was obtained from national database which routinely collects information on medication purchases from all Finnish citizens, thus not affected by recall bias.

The relationship between use of other antiarrhythmic drugs and prostate cancer has not been studied earlier. Overall, use of any antiarrhythmic drug was not associated with the risk of overall or advanced prostate cancer. An exception was sotalol, which has both K^+-channel inhibitor and beta-blocker activity. Sotalol users had decreased risk of advanced prostate cancer, and also lowered overall prostate cancer risk in long-term users. This is a novel finding. Further, inverse association with advanced prostate cancer persisted in all sensitivity analyses, suggesting that sotalol may indeed prove to be an interesting prostate cancer preventive agent. However, it should be noted that these findings could be due to chance after doing multiple comparisons for several different drugs. Therefore our results need to be validated in other studies.

In our previous study on antihypertensive drugs and prostate cancer risk within this same study population we observed a slightly increased overall prostate cancer risk, but no change in risk of advanced disease among beta-blocker users.[11] Thus it is probably not sotalol's beta-blocking properties that affect prostate cancer risk. This is supported by lack of risk association for other non-selective beta-blockers when sotalol usage has been excluded. On the other hand, beta-blockers may be beneficial in breast cancer[17,18], and sympathomimetic activity probably has a role also in prostatic carcinogenesis[19]. We observed a borderline significant decrease in risk of advanced prostate cancer in users of hydrophilic beta-blockers even after exclusion of sotalol usage. Therefore beta-blockers, particularly hydrophilic beta-blockers deserve further studies as prostate cancer preventive agents. The difference between sotalol and other beta-blockers is sotalol's function as a potassium-channel inhibitor in addition to its beta-blocker function. Potassium-channel activity and expression have been suggested to affect prostate carcinogenesis,[20] possibly by altering intracellular Ca2+ concentrations.[21] Interestingly, also digoxin's antitumor effects have been suggested to be mediated by changes in intracellular Ca2+.[2,3] Control of intracellular Ca2+ as a way to limit prostate cancer cell growth merits further study. However, the association between sotalol and advanced prostate cancer risk was not dose-dependent, and use of the other potassium-channel blocker, amiodarone, was not associated with prostate cancer risk in our study. Thus, further study is required to affirm our findings.

Our study has important strengths. We had a large population-based study population representing the entire

Finland. This allowed enough statistical power to analyze the impact of rarely used drugs, such as digoxin on prostate cancer incidence, and even on advanced prostate cancer. Our information on medication usage was obtained from a comprehensive nationwide prescription database which records information on drug purchases independent of disease status. The controls in our population were individually matched to the cases for age and residence area at the time of diagnosis to avoid confounding by these attributes. The population in Finland is racially homogenous consisting of 98% Caucasians. Thus, race is unlikely to be a confounding factor in our study.

Our study also has weaknesses that should be considered. We had no information on the antiarrhythmic drug use prior to 1995 which may lead to underestimation of cumulative duration and amount of medication use for those persons who had a long history of antiarrhythmic drug use prior to 1995. This would presumably bias our results towards the null. Indeed, when we evaluated this bias in a sensitivity analysis limited to include only the cases diagnosed during 2000–2002, *i.e.,* using the case-control pairs with longest information on medication use before the diagnosis, we found slightly decreased overall prostate cancer risk among digoxin users. This further supports the notion that very long-term digoxin use might have a prostate cancer risk decreasing effect.

We did not have information on PSA testing activity within our study population, which could have been more common among antiarrhythmic drug users than non-users, possibly creating a detection bias that elevates the observed prostate cancer risk in medication users and masks possible protective associations. We evaluated this bias by stratifying our analysis by BPH medication usage. Diagnostic work-up of BPH involves PSA testing for exclusion of prostate cancer. Thus BPH medication users present a group of PSA tested men. However, the association between antiarrhythmic drug use and prostate cancer risk was not modified by BPH medication usage, thus this detection bias is unlikely to affect our results to any great degree.

To estimate whether baseline co-morbidities were confounding the association between antiarrhythmic drug use and prostate cancer we performed sensitivity analysis stratified by use of antidiabetic drugs, cholesterol-lowering statin drugs, antihypertensive drugs and aspirin and other non-steroidal anti-inflammatory drugs. Since we found no significant interaction for any of these among the users of sotalol, it is justified to presume confounding by other conditions being negligible.

Tumor stage was known for slightly more than half of the cases (55%). The proportion of cases with missing information on stage was higher among antiarrhythmic drug users and digoxin users (48.7% and 48.5%, respectively) than among non-users of antiarrhythmic drugs (44.6%). This could have masked possible protective associations for advanced prostate cancer among users of digoxin or other antiarrhythmic drugs.

Finally, we did not have information on lifestyle factors apart from medication use, such as BMI, smoking or diet. These could have caused confounding in either direction depending on their association with antiarrhythmic medication usage.

We have shown that use of antiarrhythmic drugs in general does not associate with prostate cancer risk. Further, our study confirms that digoxin does not have a population-level prostate cancer preventive effect during a maximum exposure period of eight years. However, we cannot rule out longer term protective effects of digoxin that have been suggested by previous research. Studies with longer follow-up time will be needed. The K^+-channel inhibitor/beta-blocker sotalol showed promise for a protective effect against advanced prostate cancer. If this is confirmed in further studies, sotalol may prove to be a promising prostate cancer preventive agent.

Epidemiology

References

1. Ferlay J, Bray F, Pisani P et al. GLOBOCAN 2002: Cancer incidence, mortality and prevalence worldwide. IARC CancerBase No. 5. version 2.0. Lyon: IARC Press, 2004.
2. Yeh JY, Huang WJ, Kan SF, et al. Inhibitory effects of digitalis on the proliferation of androgen dependent and independent prostate cancer cells. *J Urol* 2001;166:1937–42.
3. McConkey DJ, Lin Y, Nutt LK, et al. Cardiac glycosides stimulate Ca2+ increases and apoptosis in androgen-independent, metastatic human prostate adenocarcinoma cells. *Cancer Res* 2000; 60:3807–12.
4. Lin H, Juang JL, Wang PS. Involvement of Cdk5/p25 in digoxin-triggered prostate cancer cell apoptosis. *J Biol Chem* 2004;279:29302–7.
5. Juang HH, Lin YF, Chang PL, et al. Cardiac glycosides decrease prostate specific antigen expression by down-regulation of prostate derived ets factor. *J Urol* 2010;184:2158–64.
6. Gayed BA, O'Malley KJ, Pilch J, et al. Digoxin inhibits blood vessel density and HIF-1a expression in castration-resistant C4-2 xenograft prostate tumors. *Clin Trans Sci* 2012;5:39–42.
7. Biggar RJ. Molecular pathways: digoxin use and estrogen-sensitive cancers–risks and possible therapeutic implications. *Clin Cancer Res* 2012;18: 2133–7.
8. Platz EA, Yegnasubramanian S, Liu JO, et al. A novel two-stage, transdisciplinary study identifies digoxin as a possible drug for prostate cancer treatment. *Cancer Discov* 2011;1:68–77.
9. Wright JL, Hansten PD, Stanford JL. Is digoxin use for cardiovascular disease associated with risk of prostate cancer?. *Prostate* 2014;74:97–102.
10. Murtola TJ, Tammela TL, Lahtela J, et al. Antidiabetic medication and prostate cancer risk: a population-based case-control study. *Am J Epidemiol* 2008;168:925–31.
11. Kemppainen KJ, Tammela TL, Auvinen A, et al. The association between antihypertensive drug use and incidence of prostate cancer in finland: a population-based case-control study. *Cancer Causes Control* 2011;22:1445–52.
12. Murtola TJ, Tammela TL, Lahtela J, et al. Cholesterol-lowering drugs and prostate cancer risk: a population-based case-control study. *Cancer Epidemiol Biomarkers Prev* 2007;16:2226–32.
13. Murtola TJ, Tammela TL, Maattanen L, et al. Prostate cancer risk among users of finasteride and alpha-blockers—a population based case-control study. *Eur J Cancer* 2007;43:775–81.
14. Teppo L, Pukkala E, Lehtonen M. Data quality and quality control of a population-based cancer registry. *Acta Oncol* 1994;33:365–9.
15. Martikainen J, Rajaniemi S. Drug reimbursement systems in EU member states, Iceland and Norway. The Social Insurance Institution, Finland, Social security and health reports 54. 2002. Available at: http://www.kela.fi/in/internet/english.nsf/

NET/100203115310PN. Accessed February 12, 2015.

16. World Health Organization. ATC/DDD index database. Available at: http://www.whocc.no/atcddd/indexdatabase/index.php?query=A10). Accessed February 12, 2015.
17. Sørensen GV, Ganz PA, Cole SW, et al. Use of β-blockers, angiotensin-converting enzyme inhibitors, angiotensin II receptor blockers, and risk of breast cancer recurrence: a danish nationwide prospective cohort study. *J Clin Oncol* 2013;31: 2265–72.
18. Ganz PA, Habel LA, Weltzien EK, et al. Examining the influence of beta blockers and ACE inhibitors on the risk for breast cancer recurrence: results from the LACE cohort. *Breast Cancer Res Treat* 2011;129:549–56.
19. Magnon C, Hall SJ, Lin J, et al. Autonomic nerve development contributes to prostate cancer progression. *Science* 2013;341:1236361
20. Ohya S, Kimura K, Niwa S, et al. Malignancy grade-dependent expression of K+-channel subtypes in human prostate cancer. *J Pharmacol Sci* 2009;109:148–51.
21. Lallet-Daher H, Roudbaraki M, Bavencoffe A, et al. Intermediate-conductance Ca2+-activated K+ channels (IKCa1) regulate human prostate cancer cell proliferation through a close control of calcium entry. *Oncogene* 2009;28:1792–806.

PUBLICATION II

Prostate cancer risk among users of digoxin and other antiarrhythmic drugs in the finnish prostate cancer screening trial

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Prostate cancer risk among users of digoxin and other antiarrhythmic drugs in the Finnish Prostate Cancer Screening Trial

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Abstract

Purpose: Long-term usage of the antiarrhythmic drug digoxin has been connected to lowered risk of prostate cancer. A recent study has suggested that beta-blockers might also have similar risk decreasing effects. We evaluated the association between use of digoxin, beta-blocker sotalol and other antiarrhythmic drugs and prostate cancer risk in a retrospective cohort study.

Methods: Our study population consisted of men in the Finnish Prostate Cancer Screening Trial during 1996 – 2012 (n=78,615). During median follow-up of 12 years, 6,639 prostate cancer cases were diagnosed. The national prescription database was the source of the information of antiarrhythmic drug purchases. Data was analyzed using Cox regression method with medication use as a time-dependent variable.

Results: No association was found for overall prostate cancer risk with antiarrhythmic drug use (HR 1.05 95% CI 0.94-1.18). Neither sotalol (HR 0.97 95% CI 0.76-1.24) nor digoxin (HR 1.01 95% CI 0.87-1.16) users had a decreased risk of prostate cancer. Similar results were obtained for high-grade (Gleason 7-10) and metastatic prostate cancer. Nevertheless, the risk estimates for Gleason 7-10 prostate cancer tended to decrease by duration of digoxin use (p for trend = 0.052), suggesting that the drug may reduce the risk in long-term usage (HR 0.71, 95% CI 0.49-1.03). In analysis stratified by screening trial arm the protective association against Gleason 7-10 disease was observed only in the screening arm (HR 0.31, 95% CI 0.12-0.84 for men who had used digoxin for five years or longer).

Conclusion: Digoxin or other antiarrhythmic drugs are not associated with any clear decrease in prostate cancer risk. However, digoxin might have a benefit in long-term use by reducing risk of high-grade disease. Further research will be needed to evaluate possible effects on prostate cancer survival.

INTRODUCTION

Despite being the most common cancer among men, prostate cancer etiology remains poorly understood. Even minor preventive effects would have major benefits to both public health and economics. The American Cancer Society estimates that in 2011, 240,890 men were diagnosed with prostate cancer and 33,720 men died because of the disease in the U.S.[1] As little as one percent reduction in population risk of prostate cancer would mean that thousands of cancers did not occur[2].

The antiarrhythmic drug digoxin has been suggested to have prostate cancer preventive effects both by *in vitro* and epidemiological studies[3,4]. In vitro digoxin inhibits plasma membrane Na+/K+ -ATPase and disarrays intracellular K+ and Ca2+ concentrations[4]. An increased Ca2+ concentration in the cell increases apoptosis[5]. In support of digoxin's beneficial effects, a large cohort study (47,884 men) recently showed decreased prostate cancer incidence among men who had used digoxin constantly for over ten years[4] and a case-control (1,001 cases and 942 controls) study reported the prostate cancer risk was decreased in digoxin users especially among men with 3 or more PSA-tests during the past five years.[3]

Beta-blockers are usually used for management of hypertension but sotalol, which is both a beta- and a K^+-channel blocker, is a common antiarrhythmic drug. It has been suggested that regular use of beta-blockers is associated with decreased risk of cancer[6]. We have previously shown that sotalol users may have lowered prostate cancer risk[7].

We analyzed how use of digoxin, sotalol or other antiarrhythmic drugs is linked with overall prostate cancer risk and with tumor characteristics at diagnosis in a cohort of men participating in the Finnish Prostate Cancer Screening Trial.

MATERIALS & METHODS

Study Population

The study cohort consisted of men randomized to the Finnish Prostate Cancer Screening Trial (FinRSPC) during 1996-1999 and followed up until the end of 2012. The FinRSPC protocol has been previously described in detail[8]. In brief, 80,456 men aged 55-67 years and living in the metropolitan areas of Tampere and Helsinki, Finland, were identified from the Population Register Center and randomized either to be screened for prostate cancer with PSA test at four-year intervals (31,866 men, the screening arm) or to be followed through the national Finnish Cancer Registry (48,278 men, the control arm).

Information on prostate cancers cases diagnosed in the study population included information on tumor Gleason grade at diagnosis, TNM stage, serum PSA value (for the screening arm) and the date and method of diagnosis.

Prostate cancer was diagnosed in 6,639 men of the study population before 2010. The method of detection was known for 6,082 cases (91.6%). Of these, 2,584 (42.5%) were detected through screening, 1,938 (31.9%) between the screening rounds, 327 (5.4%) in men invited to screening but not participating and 29 (0.5%) in autopsy. Most cases were histologically confirmed (98.1%). Other methods of diagnostic verification included clinical (0.3%) and at autopsy (1.6%). One case was only radiologically and one cytologically confirmed. The method of diagnosis was unknown in three cases.

The study was approved by the Ethics committee of the Pirkanmaa health care district, Finland (tracking number ETL 95077).

Information on medication use

Data of refunded physician-prescribed medication purchases for the entire cohort during 1995 – 2009 was collected from the nationwide prescription database of the Social Insurance Institution (SII) of Finland. SII provides reimbursements for the costs of medicine prescribed by a physician for all Finnish residents[9]. Every reimbursed purchase of a prescribed drug is registered in the database. The information in the registry includes date for each purchase, number of packages obtained, as well as the number and dosage of pills.

Information on drugs categorized as antiarrhythmic in Pharmaca Fennica, the Finnish national pharmaceutical guide was collected: amiodarone, digoxin, disopyramide, etilefrine, flecainide, quinidine, mexiletine, procainamide, propafenone and sotalol. All drugs were available for every year of the study follow-up except etilefrine (1995 – 2008) and procainamide (only 1995). The purchases of most drugs reduced significantly over time. For example, 6,110 men bought digoxin in 1997 but only 1,815 in 2009. Only purchases of flecainide increased from 92 (in 1995) to 473 (in 2009) during the study. Purchases of amiodarone and propafenone remained constant. Information on medication use was available for 78,615 men (98.1% of the entire screening trial population).

Statistical Analysis

We used Cox regression method to analyze prostate cancer risk overall, as well as by stage and Gleason grade. We performed age-adjusted and multivariable analyses (further adjustment for use of other drug groups: NSAIDs, aspirin, antidiabetic medication, statins, antihypertensives, 5alpha-reductase inhibitors and alpha-blockers). Multivariable adjusted risk estimates are reported unless otherwise stated. We analyzed class effect of antiarrhythmic drugs by comparing users of any antiarrhythmic drugs to non-users. Drug-specific effects were separately analyzed for digoxin and sotalol. Men who had used both drug groups were included in both analyses. Sensitivity analyses with further adjustment for digoxin or sotalol usage were performed to adjust for simultaneous usage.

Medication use status was updated prospectively each year of follow-up based on yearly medication purchases. Men with recorded purchases at any given year were regarded as users for that year. Users with a full year without purchases changed status into previous users. The status was allowed to change back to users if drug purchases were resumed at later point of follow-up. Never-users and all users before the first purchase were classified as non-users. Non-users were used as the reference group in all analyses.

The amount of antiarrhythmic drug use was standardized by dividing the yearly mg amount of each drug with the standard Defined Daily Dose (DDD) published in public WHO website[10]. Duration of usage was calculated as number of years with medication purchases. Intensity of use (DDDs/year) was calculated by dividing the yearly cumulative amount with the number of years of usage. In men who stopped previous usage before the end of follow-up, the cumulative amount, duration and intensity of use remained at the level reached before discontinuation

Cumulative amount (DDDs), duration (years) and intensity (DDDs/year) of medication use were also updated prospectively according to the yearly purchases. Men discontinuing previous use retained the level reached before the discontinuation. Trends in prostate cancer risk by amount, duration and intensity of the medication use were evaluated by stratifying the cohort by tertiles and repeating the analysis for each stratum. Additionally, we analyzed the trends by adding the cumulative amount/duration/intensity of use as a continuous variable into the Cox regression model. These analyses were necessary to estimate the association between prostate cancer risk and long-term drug usage, which was linked with reduced risk in the previous study[3].

Subgroup analyses were performed by stratifying the population by baseline characteristics, screening trial arm and by usage of other drug groups. We estimated effect modification by these variables on prostate cancer risk among antiarrhythmic medication users by adding interaction term with medication use into the Cox regression model. Furthermore, we estimated the effect of confounding by indication comparing digoxin users to men using other types of antiarrhythmic drugs or antihypertensive drugs.

We used X^2-test to estimate the statistical significance of differences in population characteristics by antiarrhythmic drug use.

All statistical tests are two-sided. P values 0.05 or less were considered statistically significant. IBM SPSS Statistics 22 (Chicago, IL, USA) software was used for data analyses.

RESULTS

Population Characteristics

The overall prevalence of antiarrhythmic drug use was 10.3% (8,064 men). The prevalence of digoxin use was 7.2% (5,668 men) and for sotalol 3.2% (2,540 men). Median age at baseline among never-users of antiarrhythmic drugs was 59 years. Among users the median age was slightly greater, 63 years. A similar difference was observed between ever vs. never users of digoxin and sotalol. No differences were observed in baseline PSA levels by antiarrhythmic drug use (Table 1).

During the median follow-up of 12 years 6,639 prostate cancer cases were diagnosed within the cohort. Compared to non-users of antiarrhythmic drugs, the users had lower cumulative incidence of high-grade (Gleason 7-10) tumors (42.2% vs 39.2) and metastatic disease at diagnosis (6.29% vs 5.89%, respectively) (Table 1). The incidence of high-grade disease was also slightly lower among users of digoxin (40.8% vs. 39.2% in non-users and users, respectively) and sotalol (41.9% vs. 40.2%).

The usage of other drug groups (NSAIDs, aspirin, 5alfa-reductase inhibitors, alpha-blockers, antihypertensive drugs, antidiabetic drugs and statins) was more frequent among antiarrhythmic drug users compared to the non-users (Table 1).

Antiarrhythmic drugs in general and prostate cancer

Overall prostate cancer risk was slightly elevated among current antiarrhythmic drug users compared to non-users in the age-adjusted analysis, but not in the multivariable-adjusted analysis (Table 2). No significant association was observed either for risk of high-grade or metastatic prostate cancer.

The overall prostate cancer risk increased slightly by cumulative amount and intensity of antiarrhythmic drug use, although the trend was not significant (Table 3). This trend, however, was not observed for high-grade cancer. For metastatic cancer, the risk was elevated at the beginning of usage, i.e. men whose cumulative amount was lowest, but not with continued use.

Digoxin use and prostate cancer

Overall, digoxin use was not associated with prostate cancer risk or with tumor grade or stage (Table 2). However, the association between digoxin use and prostate cancer was modified by prostate cancer screening; digoxin users had a borderline significantly decreased prostate cancer risk in the screening arm (HR 0.82, 95% CI 0.64-1.04) but not in the control arm (p for interaction = 0.052) (Table 2).

Risk estimates for high-grade and metastatic prostate cancer tended to decrease with increasing amount and duration of digoxin use (Table 3), but remained statistically non-significant. A borderline significant decrease in the risk of high-grade prostate cancer was observed among men who used digoxin for six years or longer (HR 0.71, 95% CI 0.49-1.03). A similar decrease in the risk estimate was also observed for metastatic disease in long-term users (HR 0.80, 95% CI 0.30-2.16). No clear trends in risk estimates were observed for intensity of digoxin use.

Among men in the screening arm a significant risk reduction for Gleason 7-10 prostate cancer was observed for men that have used digoxin for longer than 5 years (HR 0.31, 95% CI 0.12-0.84). Antiarrhythmic drug use in general among the same sub-cohort (screening arm, longer than 5 years of drug usage) was not associated with significant risk reduction (HR 0.64, 95% CI 0.40-1.02).

Sotalol use and prostate cancer

No risk association was observed with sotalol use for overall, high-grade and metastasized prostate cancer risks (Table 2).

The amount of sotalol usage was not associated with overall or high-grade prostate cancer risk. No significant risk difference was observed for metastatic disease, either (Table 3).

Subgroup analyses

Age at randomization modified the association between antiarrhythmic drugs and prostate cancer risk; the risk was lower in men aged 55-59 years at baseline (p for interaction = 0.001). The overall prostate cancer risk was decreased among 55-59 years old current sotalol user compared to non-users; HR 0.54, 95% CI 0.30-0.97. This difference was not seen in the older age group (p for interaction = 0.006) (Table 4).

Prostate cancer risk in digoxin users did not differ from men using other types of antiarrhythmic drugs in a sensitivity analysis limited to drug users only (HR 0.96, 95% CI 0.81-1.15). No risk difference was observed for high-grade or metastatic disease, either. Further, digoxin use was not associated with prostate cancer risk in analysis with antihypertensive drug users as the comparison group (HR 1.00, 95% CI 0.86-1.16).

Use of other medications did not modify the effect of antiarrhythmic drugs (Table 4). The sensitivity analyses to estimate the effect of simultaneous usage of digoxin and sotalol did not show any differences in prostate cancer risk estimates.

DISCUSSION

We did not find a clear association between antiarrhythmic drug usage and prostate cancer risk. Similarly, neither usage of digoxin nor sotalol had an influence on the risk. Nonetheless, the risk estimates for high-grade and metastatic prostate cancer tended to decrease by increasing cumulative amount and duration of digoxin use. However, a similar decreasing trend was observed also for antiarrhythmic drugs in general, and could thus be due to systematic differences between users and non-users of this drug group rather than due to digoxin usage. However, the risk reduction for Gleason 7-10 cancer in the screening arm was observed only for long-term users of digoxin. Thus our results lend some support for oncological benefits of long-term digoxin use, as previously reported by Platz et al in a case-control study[3].

We must consider the possibility that the observed risk associations result from confounding by indication. When digoxin users were compared to other antiarrhythmic drug users, i.e. within the group supposedly having similar indications for drug usage, no protective risk differences were observed.

Besides being used in treatment of atrial fibrillation, digoxin is also used in management of congestive heart failure. Antihypertensive drugs are also commonly used in heart failure patients. However, no risk association was found when digoxin users were compared to these antihypertensive drug users.

The Health Professionals Follow-up study demonstrated that long-term digoxin users (>10 years) had a lowered prostate cancer risk (RR 0.54 95 % CI 0.37-0.79, P-trend < 0.001)[4]. Our study cohort was larger (78,615 vs. 47,884) with more prostate cancer diagnoses (6,639 vs. 5,002) and more digoxin-users (485 vs. 243). In the previous study, only 28 men had used digoxin over ten years. We categorized the use of 6 years or more as long-term usage. Our study population consisted of 305 long-term users and 175 men that have used digoxin for over 10 years. Therefore our study had greater statistical power to study the long-term effects of these drugs. It should be noted that we observed a protective risk association for long-term digoxin use only among men under prostate cancer screening, not in the control arm. Due to widespread

PSA testing in the US, participants of the Health Professionals Follow-up study were likely in regular PSA surveillance. Thus our results are in concordance with this previous study.

Our study cohort also has some similarities to the Health Professionals study. At the beginning of the follow-up, participants were 40 – 75 years old in the US study, whereas the age of our study population was 55-67 at the start of the follow-up. Both cohorts consisted mostly of Caucasians, so ethnicity is unlikely to be a confounding factor.

Our study has some strengths. Our information on medication use was comprehensive with minimal misclassification; a possible recall bias is excluded in our study as medication purchases are recorded by the database regardless of cancer status. Another important strength of our study is the large study population consisting of men living in two metropolitan areas in Finland. The study cohort was large enough to analyze the influence of an uncommonly used drug, such as digoxin, on the risk of prostate cancer, even by disease grade and stage. In comparison to the previous US study, our information on medication use was not collected from surveys but from objectively recorded national registry data.

Some limitations should be considered. First, from the nationwide prescription database of the SII of Finland we were able to obtain the purchase information of any reimbursed drugs. Conversely, the exact indication for the purchases of the antiarrhythmic drugs was not available and thus we were not able to control for indication of drug usage in our analysis. However, evidence linking cardiac arrhythmias to prostate cancer risk is sparse. We were able control for underlying diseases indirectly by adjusting for the usage of other drug groups.

Second, we had no information on lifestyle factors. Previous studies have showed that factors such as exercise, BMI, smoking and diet might have an impact on the risk of prostate cancer[11]. These could have caused confounding in either direction depending on their distribution between the users and non-users of the antiarrhythmic drugs.

Third, we did not have information about whether the received drugs were actually used. This might have caused exposure misclassification and bias towards the null.

Conclusion

In conclusion, neither antiarrhythmic drug usage in general, nor digoxin or sotalol usage impacted the overall risk of prostate cancer. Nonetheless, our study does lend some support for the protective effect of long-term usage of digoxin, as the risk estimates for high-grade (Gleason score 7-10) cancer tended to decrease as the cumulative years of digoxin use increase. This effect was more distinct among men in the screening arm. Further studies should address whether or not digoxin use affects prostate cancer mortality.

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Disclosure

Conflicts of interest: T. J. Murtola: Lecture fee from Janssen-Cilag and MSD, K. Taari: Paid consultant for Astellas, GSK, Amgen, Pfized, Sanofi, Janssen-Cilag, employee of Medication Inc, TLJ Tammela: Paid consultant for Astellas, GSK, Pfizer, Orion Pharma and Amgen

All remaining authors have declared no conflicts of interest.

REFERENCES

1. Brawley OW (2012) Prostate cancer epidemiology in the United States. World J Urol 30:195-200

2. Hsing AW, Chokkalingam AP (2006) Prostate cancer epidemiology. Front Biosci 11:1388-413

3. Wright JL, Hansten PD, Stanford JL (2014) Is digoxin use for cardiovascular disease associated with risk of prostate cancer? Prostate 74:97-102

4. Platz EA, Yegnasubramanian S, Liu JO et al (2011) A novel two-stage, transdisciplinary study identifies digoxin as a possible drug for prostate cancer treatment. Cancer Discov 1:68-77

5. Prevarskaya N, Skryma R, Shuba Y (2004) Ca2+ homeostasis in apoptotic resistance of prostate cancer cells. Biochem Biophys Res Commun 322:1326-35

6. Monami M, Filippi L, Ungar A, et al (2013) Further data on beta-blockers and cancer risk: observational study and meta-analysis of randomized clinical trials. Curr Med Res Opin 29:369-78

7. Kaapu KJ, Ahti J, Tammela TL, Murtola TJ (2015) Sotalol, but not digoxin is associated with decreased prostate cancer risk: A population-based case-control study. Int J Cancer. 2015 February 5 [Epub ahead of print] doi:10.1002/ijc.29470

8. Kilpeläinen TP, Tammela TL, Malila N, et al (2013) Prostate Cancer Mortality in the Finnish Randomized Screening Trial. J Natl Cancer Inst 105:719-25.

9. Hemminki E, Bomann-Larsen P (1981) Drug reimbursement in four Nordic countries. Scand J Public Health 9:1-10

10. WHO Collaborating Centre for Drug Statistics Methodology. ATC/DDD Index 2013. 2013. Available from: http://www.whocc.no/atc_ddd_index/.

11. Wilson KM, Giovannucci EL, Mucci LA (2012) Lifestyle and dietary factors in the prevention of lethal prostate cancer. Asian J Androl 14:365-74

Table 1. Population characteristics

	Use of antiarrhytmic drugs			Use of digoxin			Use of sotalol		
	Never	Ever	P-value	Never	Ever	P-value	Never	Ever	P-value
N of cases	5,807	730		6,052	485		6,296	241	
Gleason 6 or less tumor	3,203 (55.1%)	419 (57.4%)		3,343 (55.3%)	279 (57.5%)		3,488 (55.4%)	134 (55.6%)	
Gleason 7-10	2,450 (42.2%)	286 (39.2%)		2,456 (40.8%)	190 (39.2%)		2,639 (41.9%)	97 (40.2%)	
Metastatic cases	365 (6.29%)	43 (5.89%)		376 (6.21%)	32 (6.60%)		392 (6.23%)	16 (6.64%)	
Median PSA-level (ng/ml)	1.07	1.07	0.49	1.07	1.08	0.59	1.08	1.01	0.28
Use of other drugs									
NSAIDs	55,664 (78.9%)	6,609 (82.0%)	0.001	57,722 (79.1%)	4,551 (80.3%)	0.039	60,131 (79.0%)	2,142 (84.3%)	0.001
Aspirin	10,894 (15.4%)	1,485 (18.4%)	0.001	11,409 (15.6%)	970 (17.1%)	0.004	11,832 (15.6%)	547 (21.5%)	0.001
Statins	28,526 (40.4%)	4,238 (53.7%)	0.001	29,905 (41.0%)	2,949 (52.0%)	0.001	31,339 (41.2%)	1,515 (59.6%)	0.001
Antidiabetic drugs	13,453 (19.1%)	2,440 (30.3%)	0.001	13,979 (19.2%)	1,914 (33.8%)	0.001	15,177 (20.0%)	716 (28.2%)	0.001
Antihypertensives	45,183 (64.0%)	7,748 (96.1%)	0.001	47,372 (64.9%)	5,559 (98.1%)	0.001	50,489 (66.4%)	2,442 (96.1%)	0.001
5alpha-reductase inhibitors	8,538 (12.1%)	1,148 (14.2%)	0.001	8,946 (12.3%)	740 (13.1%)	0.081	9,299 (12.2%)	387 (15.2%)	0.001
Alpha-blockers	18,776 (26.6%)	2,567 (31.8%)	0.001	19,643 (26.9%)	1,700 (30.0%)	0.001	20,491 (26.9%)	852 (33.5%)	0.001

Table 2. Prostate cancer risk, overall and by grade and stage in antiarrhythmic drug users

		All FinPCST participants		Screening arm	Control arm
	N	Age-adjusted analysis	Multivariable-adjusted analysis[a]	Multivariable-adjusted analysis[a,b]	Multivariable-adjusted analysis[a,b]
Overall risk		HR (95% CI)	HR (95% CI)	HR (95% CI)	HR (95% CI)
All antiarrhythmic drugs					
Users	319	1.13 (1.01-1.27)	1.05 (0.94-1.18)	0.97 (0.81-1.16)	1.09 (0.94-1.26)
Previous users	197	1.08 (0.93-1.26)	1.00 (0.86-1.17)	1.00 (0.78-1.28)	0.99 (0.81-1.21)
Digoxin					
Users	191	1.06 (0.92-1.23)	1.01 (0.87-1.16)	0.82 (0.64-1.04)	1.13 (0.94-1.35)
Previous users	135	1.09 (0.90-1.31)	1.03 (0.85-1.24)	1.08 (0.81-1.43)	0.97 (0.76-1.25)
Sotalol					
Users	63	1.05 (0.82-1.34)	0.97 (0.76-1.24)	0.88 (0.60-1.30)	1.05 (0.76-1.45)
Previous users	129	1.16 (0.96-1.41)	1.07 (0.88-1.29)	1.13 (0.84-1.54)	1.02 (0.80-1.30)
Gleason 7-10 prostate cancer risk					
All antiarrhythmic drugs					
Users	118	0.99 (0.82-1.19)	0.90 (0.74-1.08)	0.92 (0.67-1.25)	0.88 (0.70-1.11)
Previous users	100	1.17 (0.95-1.44)	1.06 (0.86-1.31)	1.23 (0.87-1.74)	0.97 (0.74-1.26)
Digoxin					
Users	73	0.94 (0.75-1.19)	0.87 (0.69-1.10)	0.67 (0.43-1.04)	0.97 (0.73-1.27)
Previous users	66	1.12 (0.86-1.46)	1.04 (0.80-1.35)	1.28 (0.85-1.93)	0.91 (0.64-1.29)
Sotalol					
Users	25	1.11 (0.75-1.65)	1.03 (0.69-1.52)	1.25 (0.69-2.26)	0.91 (0.54-1.54)
Previous users	57	1.10 (0.83-1.46)	1.00 (0.75-1.33)	1.40 (0.90-2.16)	0.81 (0.56-1.18)
Metastatic prostate cancer risk[c]					

All antiarrhythmic drugs					
Users	24	1.33 (0.88-2.01)	1.21 (0.80-1.83)	1.48 (0.71-3.07)	1.10 (0.66-1.84)
Previous users	12	1.03 (0.55-1.93)	0.94 (0.50-1.77)	0.74 (0.18-3.02)	1.00 (0.49-2.03)
Digoxin					
Users	15	1.29 (0.77-2.16)	1.14 (0.68-1.92)	1.06 (0.39-2.90)	1.18 (0.64-2.16)
Previous users	11	1.39 (0.72-2.70)	1.25 (0.64-2.44)	1.02 (0.25-4.18)	1.34 (0.63-2.85)
Sotalol					
Users	6	1.55 (0.69-3.46)	1.49 (0.67-3.35)	1.83 (0.45-7.43)	1.36 (0.50-3.65)
Previous users	5	0.87 (0.36-2.11)	0.83 (0.34-2.01)	2.23 (0.70-7.10)	0.42 (0.10-1.68)

[a] From Cox regression model adjusted for age, screening trial arm and use of cholesterol-lowering, antidiabetic and antihypertensive drugs, aspirin and other NSAIDs, and 5alpha-reductase inhibitors and alpha-blockers

[b] p for interaction in risk of prostate cancer among digoxin users by FinPCST study arm = 0.052

[c] Stage M1 at diagnosis

Table 3. Prostate cancer risk by cumulative amount, duration and intensity of antiarrhythmic drug usage

	All antiarrhythmic drugs			Digoxin			Sotalol		
	Overall PCa Risk	High-grade PCa risk	Risk of metastatic disease	Overall PCa Risk	High-grade PCa risk	Risk of metastatic disease	Overall PCa Risk	High-grade PCa risk	Risk of metastatic disease
	HR (95% CI)$_{\text{multivariable-adjusted}}$[a]	HR (95% CI)$_{\text{multivariable-adjusted}}$	HR (95% CI)$_{\text{multivariable-adjusted}}$	HR (95% CI)$_{\text{multivariable-adjusted}}$	HR (95% CI)$_{\text{multivariable-adjusted}}$	HR (95% CI)$_{\text{multivariable-adjusted}}$	HR (95% CI)$_{\text{multivariable-adjusted}}$	HR (95% CI)$_{\text{multivariable-adjusted}}$	HR (95% CI)$_{\text{multivariable-adjusted}}$
Amount of medication use[b]									
1st tertile	0.97	1.01	1.63	1.01	0.90	1.27	0.91	0.84	1.35
	(0.83-1.13)	(0.80-1.27)	(1.01-2.63)	(0.84-1.23)	(0.66-1.22)	(0.65-2.46)	(0.70-1.19)	(0.55-1.27)	(0.56-3.27)
2nd tertile	1.03	0.96	0.94	0.99	1.12	1.29	1.03	1.08	0.80
	(0.88-1.20)	(0.76-1.23)	(0.50-1.76)	(0.81-1.21)	(0.85-1.49)	(0.66-2.50)	(0.80-1.32)	(0.74-1.58)	(0.26-2.50)
3rd tertile	1.12	0.91	0.68	1.05	0.79	0.96	1.18	1.14	1.15
	(0.96-1.31)	(0.71-1.16)	(0.30-1.52)	(0.86-1.28)	(0.57-1.10)	(0.43-2.17)	(0.90-1.55)	(0.76-1.68)	(0.37-3.60)
P for trend	0.24	0.46	0.74	0.76	0.39	0.62	0.39	0.63	0.88
Duration of medication use[c]									
1st tertile	1.01	1.06	1.41	1.04	1.03	1.44	0.91	0.78	1.31

	(0.88-1.16)	(0.86-1.32)	(0.89-2.25)	(0.88-1.22)	(0.80-1.32)	(0.84-2.46)	(0.70-1.19)	(0.50-1.21)	(0.54-3.17)
2nd tertile	1.12	0.94	1.13	1.01	1.01	1.07	1.14	1.29	1.38
	(0.97-1.29)	(0.74-1.20)	(0.63-2.03)	(0.82-1.25)	(0.74-1.39)	(0.47-2.40)	(0.89-1.45)	(0.91-1.83)	(0.57-3.35)
3rd tertile	0.94	0.83	0.45	0.97	0.71	0.80	1.05	0.97	0.38
	(0.77-1.15)	(0.62-1.11)	(0.14-1.42)	(0.77-1.23)	(0.49-1.03)	(0.30-2.16)	(0.79-1.40)	(0.63-1.47)	(0.05-2.72)
P for trend	0.61	0.28	0.78	0.98	0.20	0.85	0.52	0.73	0.88
Intensity of medication use[d]									
1st tertile	1.03	1.07	1.57	0.93	0.92	1.34	1.05	0.99	1.20
	(0.87-1.21)	(0.85-1.35)	(0.93-2.65)	(0.81-1.20)	(0.68-1.24)	(0.69-2.60)	(0.81-1.37)	(0.66-1.48)	(0.45-3.22)
2nd tertile	0.85	0.68	0.85	0.79	0.67	0.92	0.85	0.71	0.61
	(0.71-1.02)	(0.51-0.91)	(0.42-1.71)	(0.61-1.01)	(0.46-0.99)	(0.38-2.22)	(0.64-1.14)	(0.44-1.15)	(0.15-2.45)
3rd tertile	1.19	1.10	0.95	1.19	1.15	1.23	1.18	1.31	1.45
	(1.03-1.36)	(0.89-1.36)	(0.52-1.74)	(1.01-1.41)	(0.89-1.49)	(0.65-2.31)	(0.93-1.50)	(0.93-1.85)	(0.60-3.52)
P for trend	0.21	0.69	0.99	0.39	0.85	0.52	0.55	0.60	0.69

[a] From Cox regression model adjusted for age, screening trial arm and use of cholesterol-lowering, antidiabetic and antihypertensive drugs, aspirin and other NSAIDs, and 5alpha-reductase inhibitors and alpha-blockers

[b] Tertile cut-points for cumulative amount of medication use: All antiarrhythmic drugs combined 1st tertile: 1-300 DDD, 2nd tertile: 301-1,350 DDD, 3rd tertile: more than 1,350 DDD; Digoxin 1st tertile: 1-200 DDD, 2nd tertile: 201-900 DDD, 3rd tertile: more than 900 DDD; Sotalol 1st tertile: 1-200 DDD, 2nd tertile: 201-1,200 DDD, 3rd tertile: more than 1,200 DDD

[c] Tertile cut-points for cumulative duration of medication use: All antiarrhythmic drugs combined 1st tertile: 1-2 years, 2nd tertile: 3-6 years, 3rd tertile: longer than 6 years; Digoxin 1st tertile: 1-2 years, 2nd tertile: 3-5 years, 3rd tertile: longer than 5 years; Sotalol 1st tertile: 1 year, 2nd tertile: 2-4 years, 3rd tertile: longer than 4 years

[d] Tertile cut-points for intensity of medication use: All antiarrhythmic drugs combined 1st tertile: 1-125 DDDs/year, 2nd tertile: 126-250 DDDs/year, 3rd tertile: more than 250 DDDs/year; Digoxin 1st tertile: 1-100 DDDs/year, 2nd tertile: 101-185 DDDs/year, 3rd tertile: more than 185 DDDs/year; Sotalol 1st tertile: 1-115 DDDs/year, 2nd tertile: 116-280 DDDs/year, 3rd tertile: more than 280 DDDs/year

Table 4. Prostate cancer risk by antiarrhythmic drug usage in subgroups stratified by baseline variables

	All Antiarrhythmic drugs	Digoxin	Sotalol
	HR (95% CI)$_{\text{multivariable-adjusted}}$[a]	HR (95% CI)$_{\text{multivariable-adjusted}}$[a]	HR (95% CI)$_{\text{multivariable-adjusted}}$[a]
Age at randomization			
55 – 59	0.84 (0.68 – 1.04)	0.90 (0.68 – 1.18)	0.54 (0.30 – 0.97)
63 – 67	1.09 (0.95 – 1.25)	0.99 (0.84 – 1.18)	1.08 (0.82 – 1.41)
P for interaction	0.001	0.143	0.006
Antiarrhythmic drug use before randomization			
No	0.96 (0.81-1.13)	0.93 (0.75-1.15)	0.99 (0.70-1.40)
Yes	1.11 (0.79-1.55)	0.96 (0.73-1.25)	0.87 (0.59-1.28)
P for interaction	0.42	0.72	0.65
NSAID usage			
Non-users	1.26 (0.95 – 1.68)	1.19 (0.84 – 1.69)	1.40 (0.75 – 2.62)
Users	1.02 (0.90 – 1.15)	0.97 (0.83 – 1.14)	0.92 (0.70 – 1.21)
P for interaction	0.843	0.474	0.401
ASA usage			
Non-users	1.08 (0.96 – 1.22)	1.04 (0.89 – 1.21)	0.92 (0.69 – 1.22)
Users	0.91 (0.66 – 1.24)	0.83 (0.54 – 1.27)	1.26 (0.74 – 2.13)
P for interaction	0.820	0.468	0.222
Antidiabetic drug usage			
Non-users	1.06 (0.93 – 1.22)	1.00 (0.83 – 1.20)	1.00 (0.75 – 1.32)
Users	1.02 (0.82 – 1.25)	1.02 (0.80 – 1.30)	0.89 (0.54 – 1.48)
P for interaction	0.155	0.446	0.434
Statin usage			
Non-users	1.13 (0.95 – 1.33)	1.11 (0.90 – 1.36)	0.95 (0.67 – 1.43)
Users	1.00 (0.86 – 1.17)	0.93 (0.76 – 1.14)	0.98 (0.71 – 1.34)
P for interaction	0.687	0.555	0.880

Antihypertensive drug usage			
Non-users	1.13 (0.61 – 2.11)	1.34 (0.50 – 3.58)	0.51 (0.13 – 2.03)
Users	1.05 (0.94 – 1.18)	1.00 (0.86 – 1.16)	1.00 (0.78 – 1.29)
P for interaction	0.222	0.883	0.528
5alpha-reductase inhibitor usage			
Non-users	1.04 (0.92 – 1.18)	1.00 (0.85 – 1.17)	0.96 (0.74 – 1.26)
Users	1.07 (0.80 – 1.43)	1.05 (0.71 – 1.54)	0.97 (0.50 – 1.87)
P for interaction	0.958	0.878	0.584
Alpha-blocker usage			
Non-users	1.05 (0.90 – 1.24)	0.95 (0.77 – 1.17)	1.04 (0.74 – 1.45)
Users	1.03 (0.88 – 1.20)	1.04 (0.85 – 1.27)	0.91 (0.63 – 1.31)
P for interaction	0.750	0.765	0.275

[a] From Cox regression model adjusted for age, screening trial arm and use of cholesterol-lowering, antidiabetic and antihypertensive drugs, aspirin and other NSAIDs, and 5alpha-reductase inhibitors and alpha-blockers

PUBLICATION III

Digoxin and prostate cancer survival in the Finnish Randomized Study of Screening for Prostate Cancer

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Digoxin and prostate cancer survival in the Finnish Randomized Study of Screening for Prostate Cancer

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Background: Protective effects have been suggested for digoxin against prostate cancer risk. However, few studies have evaluated the possible effects on prostate cancer-specific survival. We studied the association between use of digoxin or beta-blocker sotalol and prostate cancer-specific survival as compared with users of other antiarrhythmic drugs in a retrospective cohort study.

Methods: Our study population consisted of 6537 prostate cancer cases from the Finnish Randomized Study of Screening for Prostate Cancer diagnosed during 1996–2009 (485 digoxin users). The median exposure for digoxin was 480 DDDs (interquartile range 100–1400 DDDs). During a median follow-up of 7.5 years after diagnosis, 617 men (48 digoxin users) died of prostate cancer. We collected information on antiarrhythmic drug purchases from the national prescription database. Both prediagnostic and postdiagnostic drug usages were analysed using the Cox regression method.

Results: No association was found for prostate cancer death with digoxin usage before (HR 1.00, 95% CI 0.56–1.80) or after (HR 0.81, 95% CI 0.43–1.51) prostate cancer diagnosis. The results were also comparable for sotalol and antiarrhythmic drugs in general. Among men not receiving hormonal therapy, prediagnostic digoxin usage was associated with prolonged prostate cancer survival (HR 0.20, 95% CI 0.05–0.86).

Conclusions: No general protective effects against prostate cancer were observed for digoxin or sotalol usage.

Previous epidemiological studies have suggested that the antiarrhythmic drug digoxin may have prostate cancer (PCa)-protective effects especially in long-term usage (Platz *et al*, 2011; Wright *et al*, 2014; Kaapu *et al*, 2015). The proposed mechanism at the cellular level is digoxin-induced inhibition of the plasma membrane $Na+/K+$-ATPase, which elevates the intracellular Ca^{2+} concentration, enhancing apoptosis of cancer cells (McConkey *et al*, 2000; Prevarskaya *et al*, 2014). Furthermore, HIF-1α has been reported to be overexpressed in PCa cells. This overexpression might stimulate tumour growth and metastasis. Digoxin has been proposed to inhibit HIF-1α protein synthesis and the expression of HIF-1α target genes in prostate tumours (Zhang *et al*, 2008). A previous cohort study has linked use of digoxin and other HIF-1α-inhibitory drugs with delayed occurrence of castration resistance and distant metastases in PCa patients treated with androgen-deprivation therapy (Ranasinghe *et al*, 2014).

Usage of beta-blockers may be associated with decreased cancer incidence (Monami *et al*, 2013) and cancer mortality (Choi *et al*, 2014). We have previously shown in a case–control study that use of the antiarrhythmic drug sotalol, with both beta-blocker and K^+-channel inhibitor properties, decreased the risk of advanced PCa (Kaapu *et al*, 2016). Some studies also suggest that other beta-blockers may be associated with prolonged survival of PCa patients (Grytli *et al*, 2014; Lu *et al*, 2015), although conflicting results have been presented as well (Assayag *et al*, 2014; Cardwell *et al*, 2014).

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Currently, there is little knowledge about the effect of digoxin or sotalol use on PCa mortality; two published studies suggest no connection with digoxin use (Flahavan *et al*, 2014; Lip *et al*, 2015). However, digoxin use may prolong the doubling time of prostate-specific antigen (PSA) level in PCa patients (Lin *et al*, 2014). No studies have evaluated the association between other antiarrhythmic drugs and PCa mortality.

We evaluated whether the use of digoxin, sotalol or other antiarrhythmic drugs is related to PCa survival in the Finnish Randomized Study of Screening for Prostate Cancer (FinRSPC).

MATERIALS AND METHODS

Study cohort. Our study population consisted of men within FinRSPC, the largest component of the European Randomized Study of Screening for Prostate Cancer. The detailed trial protocol has been described previously (Kilpeläinen *et al*, 2013). In brief, a total of 80 458 men aged 55–67 years were identified in the years 1996–1999 and randomised to either PCa screening with a PSA test at 4-year intervals (31 866 men, the screening arm) or no intervention (48 278 men, the control arm). Prostate cancer cases diagnosed among the study population were identified from the Finnish Cancer Registry. During 1996–2009, 6537 new cases of PCa were diagnosed. Available information on cancer cases included the Gleason grade, TNM stage, primary treatment (surgery, radiation therapy, endocrine treatment or surveillance) and the serum PSA concentration. Each case was categorised as either low/medium risk or high risk according to the criteria of the European Association of Urology.

Causes of death among the study population in 1996–2012 were obtained from Statistics Finland, which has been found to be a reliable source of information by the FinRSPC cause-of-death committee (Mäkinen *et al*, 2008). In this study, deaths where PCa (ICD-10 code C61) was recorded as the primary cause of death were considered as PCa deaths. Cases with ICD-code C61 as the intermediate or contributory cause of death were analysed separately for PCa-related mortality.

The study was approved by the Ethics committee of the Pirkanmaa health-care district, Finland (tracking number R10167).

Information on medication use. The information on antiarrhythmic drug purchases was collected from the reimbursement database of the Social Insurance Institution of Finland (SII). The database includes the information on physician-prescribed medication purchases during 1995–2009. This linkage was based on the unique personal identification number assigned for all Finnish residents. The database contains records of the date, the number of packages acquired and the number and dosage of the pills for each purchase.

All Finnish residents are entitled to a reimbursement provided by the SII for every physician-prescribed drug purchase in the outpatient setting (Hemminki and Bomann-Larsen, 1981). The database covers all antiarrhythmic drugs, including amiodarone, digoxin, disopyramide, etilefrine, flecainide, quinidine, mexiletine, procainamide, propafenone and sotalol. Additional information was obtained concerning use of statins, antidiabetic medication (oral drugs and insulins), antihypertensive medication (beta-blockers, ACE inhibitors/ATII receptor blockers, calcium-channel blockers, diuretics and other types of drugs, such as methyldopa and clonidine), aspirin and other NSAIDs, 5-alpha-reductase inhibitors and alpha-blockers. The database does not cover over-the-counter medication purchases or the drugs used by hospital inpatients.

Statistical analysis. Differences in the baseline characteristics of ever- *vs* never-users of digoxin and sotalol were compared separately using the chi-square test (categorical variables) and the Mann–Whitney *U*-test (continuous variables).

The analysis was limited to include only men who have used some antiarrhythmic drug during the study period to minimise the effects of confounding by indication. The association between usage of digoxin and sotalol and risk of PCa-specific death was estimated using the Cox regression model. Follow-up started at PCa diagnosis. The analysis was conducted separately for prediagnostic and postdiagnostic use of medication.

Antiarrhythmic drug usage before PCa diagnosis was analysed as a time-independent variable fixed at baseline. Participants using medication at the time of diagnosis were classified as active users. If the medication had been used previously but not during the year of diagnosis, the participant was classified as a previous user. Active users and previous users were also combined into one category called 'any users'.

Antiarrhythmic drug usage after PCa was analysed as a time-dependent variable. The medication use status was updated each year, based on yearly medication purchases during the follow-up. All participants were categorised as non-users until the first medication purchase. At the first purchase, the exposure status changed to user. Men who discontinued previous drug purchases remained in the category of any users to minimise bias owing to selective discontinuation of drug usage during the terminal phases of cancer.

We used three differently adjusted regression models: (1) age-adjusted (2) additionally adjusted for tumour risk group and (3) multivariable-adjusted (further adjustment for FinRSPC trial arm and use of other drugs during the study period: drugs used for benign prostatic hyperplasia, diabetes, hypercholesterolemia or hypertension and aspirin and other NSAIDs). To avoid over-adjustment of the analysis, we did not adjust for PCa treatment, as the treatment depends on patient age, tumour characteristics and co-morbidities, all of which were adjusted for, and the effect of drug use may occur through tumour characteristics.

The annual amount of medication use was estimated by adding together the milligram amount of all purchases of a given drug (dosage multiplied by the number of pills) during the year. We standardised the amount of usage between different antiarrhythmic drugs by dividing the yearly milligram amount with the drug-specific average defined daily dose (DDD) published by WHO (2015). Intensity of drug use (DDDs per year) was calculated by dividing the yearly cumulative amount with the number of years of usage.

The amount (DDDs), duration (years) and intensity (DDDs per years) of postdiagnostic antiarrhythmic drug use were also time-dependent variables, which were updated by recorded medication purchases during each year of follow-up. At discontinuation, cumulative medication use remained at the reached level.

We evaluated survival trends by amount, duration and intensity of either digoxin or sotalol use by dividing the cohort into two subgroups according to the median of cumulative amount/duration/intensity of drug use. The over-median and under-median subgroups were compared with the users of other antiarrhythmic drugs.

Effect modification by age, tumour characteristics, screening trial arm, usage of other drug groups and primary treatment was evaluated in subgroup analyses stratified according to these variables. In the subgroup analyses, non-users were used as a reference. Prediagnostic and postdiagnostic antiarrhythmic drug usages among these subgroups were analysed separately. The statistical significance of effect modification was evaluated by adding an interaction term to the Cox regression model between the variable of interest and medication use.

Several sensitivity analyses were performed to characterise the association between digoxin use and PCa-specific survival. The impact of medication use during the final years of life was evaluated in a lag time analysis, where exposure was lagged to occur 1–3 years later than the actual purchases. Possible confounding owing to background variables was controlled by

calculating a propensity score, as described previously (Rosenbaum and Rubin, 1984), and stratifying the analysis according to the median of the propensity score. In short, antiarrhythmic drug use was analysed as the dependent variable using the logistic regression method. The explanatory variables were age at diagnosis, use of other drugs and the tumour risk group. Propensities from each background variable were summed together to form a total propensity score, which was then used to stratify the population. Competing risk regression analyses with non-cancer deaths as the competing risk were carried out according to the method described by Fine and Gray (1999) in order to compare the risks of prostate cancer death among users of digoxin and users of sotalol to men using other types of antiarrhythmic drugs.

All the statistical tests mentioned above are two-sided. *P*-values of $\leqslant 0.05$ were considered statistically significant. IBM SPSS Statistics 22 (Chicago, IL, USA) software was used for data analyses.

RESULTS

Population characteristics. In the study population of 6537 PCa cases, the median age at diagnosis was 63 years among prediagnostic ever- and never-users of antiarrhythmic drugs, as well as among digoxin and sotalol users. In total, 730 men (11.2%) had used antiarrhythmic drugs during the follow-up, 485 (7.4%) had used digoxin and 241 (3.7%) sotalol. The median exposures to digoxin and sotalol were 480 and 380 DDDs (ranges 100–1400 and 50–1500 DDDs), respectively. During the median follow-up of 7.5 years after PCa diagnosis, 1861 (28.5%) subjects died, 617 (9.4%) with PCa as the underlying cause of death, including 70 men with any antiarrhythmic drug use, 48 men with digoxin use and 26 with sotalol use.

Among ever-users of antiarrhythmic drugs, the proportion of men with Gleason 7–10 cancer was slightly lower compared with never-users (39.4% *vs* 42.2%). Also the prevalence of Gleason 8–10 PCa was lower among the users (12.2% *vs* 14.1%). The same trend was observed between ever- and never-users of digoxin or sotalol (39.2% *vs* 42.0% and 40.6% *vs* 42.0%, respectively). The proportion of metastatic cases did not vary by antiarrhythmic drug usage (Table 1).

The usage of other drug groups (NSAIDs, aspirin, 5-alpha-reductase inhibitors, alpha-blockers, antihypertensive drugs, anti-diabetic drugs and statins) was generally more frequent among the antiarrhythmic drug users compared with the non-users (Table 1).

Antiarrhythmic drug use before prostate cancer diagnosis. Digoxin use was not significantly associated with the risk of PCa death (age-adjusted HR 1.33, 95% CI 0.99–1.77 and HR 1.53, 95% CI 0.88–2.65 for any use and current use, respectively; Table 2). Further adjustment for tumour risk group and use of other medications did not change the result (Figure 1). Non-significantly increased hazard ratios were observed among cases where the cumulative amount, duration or intensity of digoxin usage was above the median (Table 2).

Prediagnostic sotalol usage did not affect the risk of PCa death and no clear risk trends were observed by cumulative usage (Table 2).

Antiarrhythmic drug use after prostate cancer diagnosis. Post-diagnostic digoxin usage was not significantly associated with PCa survival (age-adjusted HR 1.19, 95% CI 0.72–1.97 and HR 1.02, 95% CI 0.60–1.87 for any and current use, respectively; Table 3). Again, further model adjustment did not change the result. No consistent survival differences were observed by cumulative amount and duration of postdiagnostic digoxin use (Table 3).

Postdiagnostic usage of sotalol was generally not significantly associated with the risk of PCa death (multivariable-adjusted HR 1.53, 95% CI 0.78–2.98 for any use; Table 3). Only men who had discontinued sotalol usage had an elevated risk of PCa death (HR 2.73, 95% CI 1.28–5.84). Risk increases were observed only in short-term use at low cumulative doses and low intensity and were no longer significant after adjustment for other prognostic factors.

Subgroup analyses. Use of ADT as primary treatment of PCa did not modify the effect of digoxin (*P* for interaction 0.60), although a significant risk decrease was observed among men not receiving ADT and using digoxin before diagnosis (HR 0.20, 95% CI 0.048–0.86).

The risk of PCa death was neither lowered nor elevated in the other analysed subgroups for digoxin use before diagnosis (Figure 2) or postdiagnosis (Figure 3).

Sensitivity analyses. The risk of PCa death was compared between all antiarrhythmic drug users and non-users to see whether there is general risk variance associated with the usage. When men with any antiarrhythmic drug usage before PCa diagnosis were compared with never-users, no risk difference was observed (HR 1.16, 95% CI 0.82–1.65). The results were similar for men with any antiarrhythmic drug usage after the diagnosis (HR 0.94, 95% CI 0.61–1.44). Furthermore, digoxin users were compared with non-users of antiarrhythmic drugs. We found no material survival association for digoxin use before (HR 1.22, 95% CI 0.87–1.72) or after (HR 1.09, 95% CI 0.72–1.65) the diagnosis. Further adjustment for primary and secondary PCa treatment did not modify the main results.

In a separate analysis, we used antihypertensive drug users as the reference group, because these drugs are often used in the management of cardiac insufficiency, which is also a common indication for digoxin use. There was no risk association observed in this analysis, neither for prediagnostic (HR 1.11, 95% CI 0.70–1.74) nor for postdiagnostic drug usage (HR 0.95, 95% CI 0.55–1.62).

No risk association was seen for PCa-related deaths (HR 0.92, 95% CI 0.54–1.56 for prediagnostic and HR 1.00, 95% CI 0.60–1.68 for postdiagnostic digoxin usage).

Digoxin usage was not associated with PCa death in lag-time analyses either: the risk estimate in the analysis with a 1-year lag was 1.40, 95% CI 0.86–2.28 and in the 3-year lag time analysis 1.34, 95% CI 0.83–2.19.

In an analysis stratified by the median of the propensity scores, the effects of digoxin use were comparable among men with low and high propensity for antiarrhythmic drug use (usage before diagnosis HR 1.72 95% CI 0.85–3.46 and 1.45 95% CI 0.81–2.59; usage after diagnosis HR 0.79 95% CI 0.30–2.12 and 1.26 95% CI 0.67–2.39, respectively). The findings for sotalol were similar. Further, digoxin or sotalol uses were not associated with the risk of PCa death in an analysis adjusted for the propensity score.

Digoxin use, both before and after PCa diagnosis, was not associated with risk of PCa death when non-cancer deaths were analysed as a competing cause of death (HR 1.03, 95% CI 0.72–1.07 and HR 0.85, 95% CI 0.60–1.22, respectively).

Overall risk of death and death owing to causes other than prostate cancer by digoxin and sotalol use are reported in Supplementary Table S1. Digoxin users were at greater risk of dying from non-PCa causes compared with other antiarrhythmic drug users, whereas the risk was lowered among sotalol users. Furthermore, we performed a Cox regression that included only those variables that showed a significant association with the risk of PCa death in crude analyses. Results were comparable to the main analysis (Supplementary Table S2).

DISCUSSION

Our study found no significant association between PCa survival and digoxin or sotalol usage. The timing of the drug usage did not affect the results, as no difference was observed between survival estimates of prediagnostic and postdiagnostic digoxin usage. No

dose–response was found in the risk by the cumulative amount, duration or intensity of digoxin use. Furthermore, the results did not differ between men in the screening and control arms. Thus our results do not support the PCa-protective effects of this antiarrhythmic agent.

A previous cohort study including 5732 PCa patients reported that digoxin usage at PCa diagnosis did not associate with PCa survival (Flahavan *et al*, 2014). Our findings are in concordance with the results reported previously, and some new aspects are considered. We analysed prediagnostic and postdiagnostic drug

Table 1. Population characteristics

	Use of antiarrhytmic drugs			Use of digoxin			Use of sotalol		
	Never	Ever	*P*-value	Never	Ever	*P*-value	Never	Ever	*P*-value
No. of cases	5807	730		6052	485		6296	241	
Gleason grade			0.23			0.35			0.56
⩽6	3205 (55.2%)	419 (57.5%)		3345 (55.3%)	279 (57.6%)		3490 (55.4%)	134 (55.6%)	
7	1632 (28.1%)	198 (27.2%)		1703 (28.1%)	127 (26.2%)		1760 (28.0%)	70 (29.0%)	
⩾8	818 (14.1%)	89 (12.2%)		844 (13.9%)	63 (13.0%)		879 (14.0%)	28 (11.6%)	
Information unknown	152 (2.6%)	22 (3.2%)		160 (2.6%)	14 (3.1%)		167 (2.6%)	9 (3.7%)	
Tumour stage at diagnosis			0.26			0.55			0.76
Localised	5300 (91.3%)	676 (92.6%)		5531 (91.4%)	445 (91.8%)		5755 (91.4%)	221 (91.7%)	
Metastatic cases	365 (6.3%)	43 (5.9%)		376 (6.2%)	32 (6.6%)		392 (6.2%)	16 (6.6%)	
The last observed PSA value	(7.00) 7.30	(7.00) 7.40	0.91	(7.00) 7.30	(7.15) 7.75	0.50	(7.00) 7.30	(7.00) 7.50	0.90
Use of other drugs									
NSAIDs	5009 (86.3%)	627 (85.9%)	0.79	5226 (86.4%)	410 (84.5%)	0.26	5432 (86.3%)	204 (84.6%)	0.47
Aspirin	773 (13.3%)	115 (15.8%)	0.070	822 (13.6%)	66 (13.6%)	0.99	842 (13.4%)	46 (19.1%)	0.011
Statins	2641 (45.5%)	418 (57.3%)	<0.001	2797 (46.2%)	262 (54.0%)	0.001	2903 (46.1%)	156 (64.7%)	<0.001
Antidiabetic drugs	1072 (18.5%)	202 (27.7%)	<0.001	1120 (18.5%)	154 (31.8%)	<0.001	1214 (19.3%)	60 (24.9%)	0.031
Antihypertensives	4034 (69.5%)	714 (97.8%)	<0.001	4269 (70.5%)	479 (98.8%)	<0.001	4511 (71.6%)	237 (98.3%)	<0.001
5-alpha-reductase inhibitors	804 (13.8%)	104 (14.2%)	0.77	844 (13.9%)	64 (13.2%)	0.65	873 (13.9%)	35 (14.5%)	0.77
Alpha-blockers	2669 (46.0%)	363 (49.7%)	0.055	2794 (46.2%)	238 (49.1%)	0.22	2908 (46.2%)	124 (51.5%)	0.11
Primary treatment									
Radical prostatectomy	1535 (26.4%)	117 (16.0%)	<0.001	1589 (26.3%)	63 (13.0%)	<0.001	1614 (25.6%)	38 (15.8%)	0.001
Radiation therapy	2069 (35.6%)	306 (41.9%)	0.002	2170 (35.9%)	205 (42.3%)	0.009	2266 (36.0%)	109 (45.2%)	0.013
Hormonal therapy	2328 (40.1%)	341 (46.7%)	0.001	2428 (40.1%)	241 (49.7%)	<0.001	2559 (40.6%)	110 (45.6%)	0.12
Active surveillance	1016 (17.5%)	136 (18.6%)	0.44	1061 (17.5%)	91 (18.8%)	0.48	1111 (17.7%)	41 (17.0%)	0.80

Abbreviations: NSAID = non-steroidal anti-inflammatory drug; PSA = prostate-specific antigen.

Table 2. Prostate cancer-specific survival among men using digoxin and sotalol before prostate cancer diagnosis as compared with other antiarrhythmic drug users in the cohort of 6537 prostate cancer cases diagnosed in the Finnish Randomized Study of Prostate Cancer Screening

	Digoxin				Sotalol			
	N	Age-adjusted HR (95% CI)	Multivariable-adjusted1[a] HR (95% CI)	Multivariable-adjusted2[b] HR (95% CI)	*N*	Age-adjusted HR (95% CI)	Multivariable-adjusted1[a] HR (95% CI)	Multivariable-adjusted2[b] HR (95% CI)
Prediagnostic usage								
None	396	Ref.	Ref.	Ref.	525	Ref.	Ref.	Ref.
Any	334	1.33 (0.99–1.77)	1.38 (0.86–2.22)	1.21 (0.71–2.05)	205	1.07 (0.80–1.43)	1.04 (0.62–1.75)	1.12 (0.63–1.98)
Current user	191	1.53 (0.88–2.65)	1.33 (0.76–2.31)	1.00 (0.56–1.80)	63	0.93 (0.40–2.16)	0.80 (0.34–1.87)	0.82 (0.34–1.97)
Previous user	143	1.69 (0.92–3.11)	1.46 (0.79–2.69)	1.57 (0.84–2.95)	142	1.17 (0.64–2.11)	1.12 (0.66–2.17)	1.16 (0.63–2.15)
Cumulative DDD amount[c]								
Under median	168	1.52 (0.85–2.72)	1.31 (0.73–2.35)	0.99 (0.52–1.88)	105	1.04 (0.52–2.04)	0.98 (0.50–1.94)	0.98 (0.47–2.03)
Over median	166	1.67 (0.94–2.95)	1.45 (0.81–2.58)	1.56 (0.84–2.91)	100	1.13 (0.57–2.23)	1.10 (0.55–2.16)	1.38 (0.64–2.97)
Cumulative years of usage[d]								
Under median	176	1.62 (0.93–2.81)	1.35 (0.78–2.35)	1.05 (0.58–1.90)	121	1.12 (0.61–2.06)	1.05 (0.57–1.93)	1.08 (0.57–2.03)
Over median	158	1.55 (0.84–2.86)	1.42 (0.76–2.65)	1.54 (0.77–3.05)	84	1.01 (0.46–2.24)	1.02 (0.46–2.26)	1.17 (0.53–2.59)
Intensity of use (DDDs per year)[e]								
Under median	174	1.60 (0.90–2.83)	1.46 (0.82–2.60)	1.10 (0.57–2.11)	103	0.86 (0.41–1.81)	0.85 (0.40–1.80)	0.84 (0.39–1.82)
Over median	160	1.59 (0.89–2.84)	1.30 (0.72–2.34)	1.32 (0.71–2.47)	102	1.31 (0.70–2.46)	1.21 (0.65–2.28)	1.18 (0.58–2.42)

Abbreviations: CI = confidence interval; DDD = defined daily dose; HR = hazard ratio.
[a]From Cox regression model adjusted for age and the tumour risk group.
[b]From Cox regression model adjusted for age, screening trial arm and use of cholesterol-lowering, antidiabetic and antihypertensive drugs, aspirin and other NSAIDs and 5-alpha-reductase inhibitors and alpha-blockers and additionally for the tumour risk group.
[c]Median for cumulative amount of medication use: Digoxin: 550 DDD; Sotalol 550 DDD.
[d]Median for cumulative duration of medication use: Digoxin: 3 years; Sotalol 3 years.
[e]Median for intensity of medication use: Digoxin: 175 DDDs per year; Sotalol 192 DDDs per year.

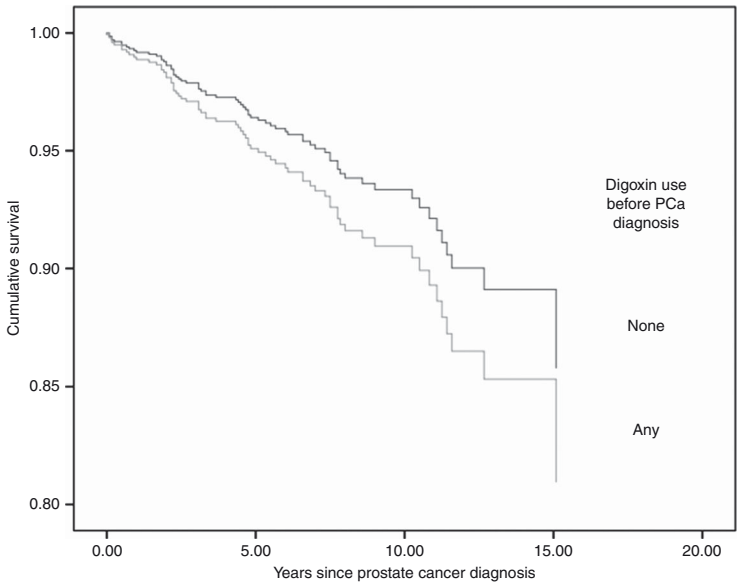

Figure 1. Kaplan–Meier plot for prostate cancer-specific survival by digoxin use before diagnosis among men using any antiarrhythmic drugs between 1995 and 2009. Cohort of 6537 prostate cancer cases diagnosed in FinRSPC.

Table 3. Prostate cancer-specific survival among men using digoxin and sotalol after prostate cancer diagnosis as compared with other antiarrhythmic drug users in the cohort of 6537 prostate cancer cases diagnosed in the Finnish Randomized Study of Prostate Cancer Screening

	Digoxin			Sotalol		
	Age-adjusted HR (95% CI)	Multivariable-adjusted1[a] HR (95% CI)	Multivariable-adjusted2[b] HR (95% CI)	Age-adjusted HR (95% CI)	Multivariable-adjusted1[a] HR (95% CI)	Multivariable-adjusted2[b] HR (95% CI)
Postdiagnostic usage						
None	Ref.	Ref.	Ref.	Ref.	Ref.	Ref.
Any	1.19 (0.72–1.97)	1.14 (0.69–1.88)	1.00 (0.59–1.71)	1.56 (0.83–2.92)	1.35 (0.72–2.53)	1.53 (0.78–2.98)
Current user	1.02 (0.60–1.87)	0.95 (0.52–1.74)	0.81 (0.43–1.51)	0.73 (0.23–2.34)	0.67 (0.21–2.15)	0.80 (0.25–2.64)
Previous user	1.62 (0.78–3.36)	1.62 (0.79–3.36)	1.42 (0.64–3.18)	2.56 (1.24–5.29)	2.08 (1.00–4.32)	2.73 (1.28–5.84)
Cumulative DDD amount[c]						
Under median	1.46 (0.82–2.59)	1.42 (0.80–2.52)	1.23 (0.67–2.23)	2.37 (1.20–4.64)	1.88 (0.96–3.71)	2.04 (0.99–4.23)
Over median	0.82 (0.37–1.83)	0.76 (0.34–1.71)	0.59 (0.24–1.43)	0.57 (0.14–2.35)	0.55 (0.13–2.29)	0.69 (0.16–2.91)
Cumulative years of usage[d]						
Under median	1.43 (0.84–2.44)	1.40 (0.82–2.39)	1.22 (0.70–2.15)	2.06 (1.01–4.16)	1.67 (0.82–3.40)	1.88 (0.89–3.94)
Over median	0.55 (0.17–1.79)	0.48 (0.15–1.59)	0.31 (0.081–1.17)	0.90 (0.28–2.90)	0.85 (0.26–2.74)	0.96 (0.29–3.21)
Intensity of use (DDDs per year)[e]						
Under median	1.39 (0.75–2.57)	1.35 (0.73–2.51)	1.24 (0.66–2.34)	2.29 (1.09–4.81)	1.80 (0.85–3.80)	1.84 (0.84–4.06)
Over median	0.99 (0.50–1.97)	0.93 (0.47–1.84)	0.71 (0.33–1.50)	0.95 (0.34–2.63)	0.90 (0.32–2.49)	1.15 (0.40–3.28)

Abbreviations: CI = confidence interval; DDD = defined daily dose; HR = hazard ratio.
[a]From Cox regression model adjusted for age and the tumour risk group.
[b]From Cox regression model adjusted for age, screening trial arm and use of cholesterol-lowering, antidiabetic and antihypertensive drugs, aspirin and other NSAIDs and 5-alpha-reductase inhibitors and alpha-blockers.
[c]Median for cumulative amount of medication use: Digoxin: 450 DDD; Sotalol 600 DDD.
[d]Median for cumulative duration of medication use: Digoxin: 3 years; Sotalol 2 years.
[e]Median for intensity of medication use: Digoxin: 150 DDDs per year; Sotalol 215 DDDs per year.

usage separately, providing new information on the possible importance of the timing of drug usage. The median follow-up time in our study was 7.5 years, while in the previous study it was 4.3 years (Flahavan *et al*, 2014). This increase in the median follow-up time is important when studying PCa death as an end point, as PCa often has a good long-term survival.

The association between digoxin usage and PCa risk has been more comprehensively studied than PCa survival. Nevertheless, incongruous results have been reported. Platz *et al* (2011) reported digoxin users having a lowered PCa risk compared with non-users in the Health Professionals Follow-up Study. The risk decrease was more distinct among men who had used digoxin for >10 years.

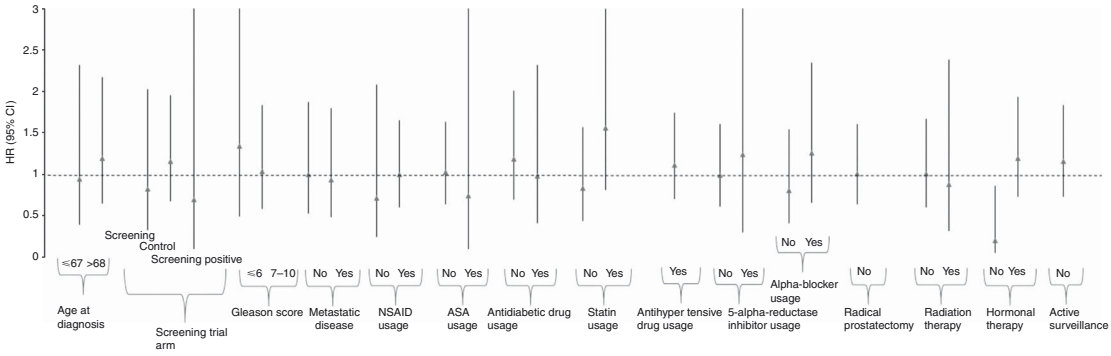

Figure 2. Subgroup analyses for men using digoxin before PCa diagnosis.

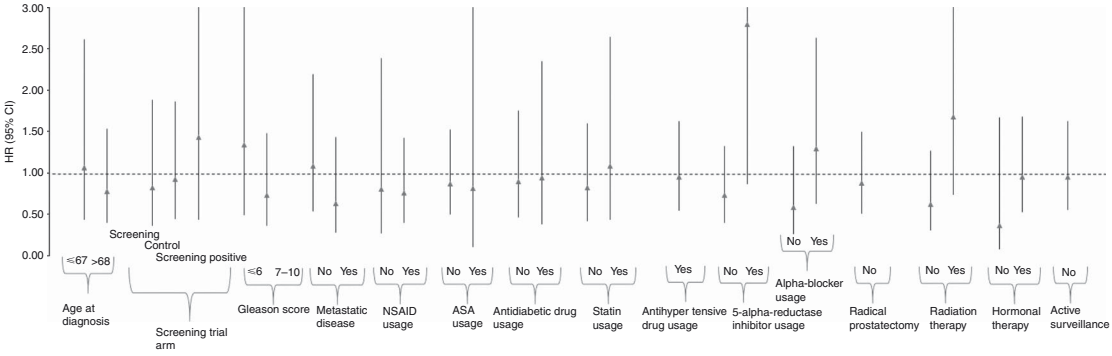

Figure 3. Subgroup analyses for men using digoxin after PCa diagnosis.

We have previously demonstrated in this study population that digoxin use may be linked with a lower risk of Gleason 7–10 PCa, specifically in men under systematic PCa screening (Kaapu *et al*, 2015). The current study shows that this possible benefit in PCa risk does not translate into improved disease-specific survival.

The only subgroup in the present study where a possible protective effect of digoxin was observed was the men who did not receive ADT as the primary treatment choice. Although the interaction term was non-significant, this suggests that ADT may modify the effects of digoxin in PCa patients. Our results do not support the previous study reporting digoxin and other HIF-1α inhibitors to enhance the efficacy of ADT (Ranasinghe *et al*, 2014). On the other hand, digoxin is a phytoestrogen affecting the estrogen receptor (Rifka *et al*, 1978). Thus the protective effects could be diluted in men managed with ADT but observed in men managed otherwise.

The decreased risk of advanced PCa observed among sotalol users in our previous study (Kaapu *et al*, 2015) did not translate into a survival benefit in the present study. Additionally, our recent cohort study (Kaapu *et al*, 2016) lacked this association and therefore we must consider the possible protective effects of sotalol usage in relation to prostate cancer death as uncertain.

Several strengths can be identified in our study. Men living in two different metropolitan areas in Finland comprised a comprehensive and representative study population. The study cohort enabled us to assess reliably the effects of relatively infrequent antiarrhythmic agents. Furthermore, information on medication use was collected from a national prescription database, thus allowing us to evaluate both prediagnostic and postdiagnostic drug usage. Recall bias was avoided, as the information on medication use was not self-reported; the database records medication purchases regardless of cancer status. In addition, information on the treatment and characteristics of the cancer was available from medical records.

Analyses on the risk of death among digoxin users are easily influenced by competing causes of death as the drug is used in the management of atrial fibrillation and cardiac insufficiency, both of which are strongly associated with cardiovascular diseases. This was demonstrated by the increased risk of non-PCa death among digoxin users. To minimise the possibility of confounding by indication, users of other antiarrhythmic drugs were used as a reference group. In the multivariable-adjusted analyses, the influence of tumour risk group and usage of other medication were considered. Furthermore, we were able to evaluate the role of screening in the survival association, as men in the screening arm and in the control arm were analysed separately. Additionally, performing the analysis with competing risk regression did not change the result.

A few limitations should be considered. The indications for antiarrhythmic drugs prescribed to men in the study were not available. Most other diseases among the men could be adjusted for in the multivariable analyses as described above, but no information on untreated chronic conditions was available. Furthermore, only 48 digoxin users died of PCa. Thus our analysis was probably underpowered to detect small differences in PCa survival.

CONCLUSION

We found no clear association between digoxin or sotalol usage and PCa-specific survival.

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CONFLICT OF INTEREST

TJ Murtola: lecture fee from Janssen-Cilag and MSD; K Taari: lecture fee GSK, paid consultancy for Abbvie, employee of Medivation, participation in the International Meeting with sponsors Astellas and Orion; TLJ Tammela: paid consultant for Astellas, GSK, Pfizer, Orion and Amgen; A Auvinen: lecture fee from MSD, paid consultancy for Epid Research. The other authors declare no conflict of interest.

REFERENCES

Assayag J, Pollak MN, Azoulay L (2014) Post-diagnostic use of beta-blockers and the risk of death in patients with prostate cancer. *Eur J Cancer* **50**: 2838–2845.

Cardwell CR, Coleman HG, Murray LJ, O'Sullivan JM, Powe DG (2014) Beta-blocker usage and prostate cancer survival: a nested case-control study in the UK Clinical Practice Research Datalink cohort. *Cancer Epidemiol* **38**: 279–285.

Choi CH, Song T, Kim TH, Choi JK, Park JY, Yoon A, Lee YY, Kim TJ, Bae DS, Lee JW, Kim BG (2014) Meta-analysis of the effects of beta blocker on survival time in cancer patients. *J Cancer Res Clin Oncol* **140**: 1179–1188.

Criteria of European Association of Urology for prostate cancer risk groups (updated Mar 2015; cited 9 Dec 2015). Available from http://uroweb.org/wp-content/uploads/EAU-Guidelines-Prostate-Cancer-2015-v2.pdf.

Fine JP, Gray RJ (1999) A proportional hazards model for the subdistribution of a competing risk. *J Am Stat Assoc* **94**: 496–509.

Flahavan EM, Sharp L, Bennett K, Barron TI (2014) A cohort study of digoxin exposure and mortality in men with prostate cancer. *BJU Int* **113**: 236–245.

Grytli HH, Fagerland MW, Fossa SD, Tasken KA (2014) Association between use of beta-blockers and prostate cancer-specific survival: a cohort study of 3561 prostate cancer patients with high-risk or metastatic disease. *Eur Urol* **65**: 635–641.

Hemminki E, Bomann-Larsen P (1981) Drug reimbursement in four Nordic countries. *Scand J Soc Med* **9**: 1–10.

Kaapu KJ, Ahti J, Tammela TL, Auvinen A, Murtola TJ (2015) Sotalol, but not digoxin is associated with decreased prostate cancer risk: a population-based case-control study. *Int J Cancer* **137**: 1187–1195.

Kaapu KJ, Murtola TJ, Määttänen L, Talala K, Taari K, Tammela TLJ, Auvinen A (2016) Prostate cancer risk among users of digoxin and other antiarrhythmic drugs in the Finnish Prostate Cancer Screening Trial. *Cancer Causes Control* **27**: 157–164.

Kilpeläinen TP, Tammela TL, Malila N, Hakama M, Santti H, Määttänen L, Stenman UH, Kujala P, Auvinen A (2013) Prostate cancer mortality in the Finnish Randomized Screening Trial. *J Natl Cancer Inst* **105**: 719–725.

Lin J, Zhan T, Duffy D, Hoffman-Censits J, Kilpatrick D, Trabulsi EJ, Lallas CD, Chervoneva I, Limentani K, Kennedy B, Kessler S, Gomella L, Antonarakis ES, Carducci MA, Force T, Kelly WK (2014) A pilot phase II Study of digoxin in patients with recurrent prostate cancer as evident by a rising PSA. *Am J Cancer Ther Pharmacol* **2**: 21–32.

Lip S, Carlin C, McCallum L, Touyz RH, Dominiczak AF, Padmanabhan S (2015) Incidence and prognosis of cancer associated with digoxin and common antihypertensive drugs. *J Hypertens* **33**(Suppl 1): E45.

Lu H, Liu X, Guo F, Tan S, Wang G, Liu H, Wang J, He X, Mo Y, Shi B (2015) Impact of beta-blockers on prostate cancer mortality: a meta-analysis of 16,825 patients. *Onco Targets Ther* **8**: 985–990.

Mäkinen T, Karhunen P, Aro J, Lahtela J, Määttänen L, Auvinen A (2008) Assessment of causes of death in a prostate cancer screening trial. *Int J Cancer* **122**: 413–417.

McConkey DJ, Lin Y, Nutt LK, Ozel HZ, Newman RA (2000) Cardiac glycosides stimulate Ca2+ increases and apoptosis in androgen-independent, metastatic human prostate adenocarcinoma cells. *Cancer Res* **60**: 3807–3812.

Monami M, Filippi L, Ungar A, Sgrilli F, Antenore A, Dicembrini I, Bagnoli P, Marchionni N, Rotella CM, Mannucci E (2013) Further data on beta-blockers and cancer risk: observational study and meta-analysis of randomized clinical trials. *Curr Med Res Opin* **29**: 369–378.

Platz EA, Yegnasubramanian S, Liu JO, Chong CR, Shim JS, Kenfield SA, Stampfer MJ, Willett WC, Giovannucci E, Nelson WG (2011) A novel two-stage, transdisciplinary study identifies digoxin as a possible drug for prostate cancer treatment. *Cancer Discov* **1**: 68–77.

Prevarskaya N, Skryma R, Shuba Y (2014) Ca2+ homeostasis in apoptotic resistance of prostate cancer cells. *Biochem Biophys Res Commun* **322**: 1326–1335.

Ranasinghe WK, Sengupta S, Williams S, Chang M, Shulkes A, Bolton DM, Baldwin G, Patel O (2014) The effects of nonspecific HIF1α inhibitors on development of castrate resistance and metastases in prostate cancer. *Cancer Med* **3**: 245–251.

Rifka SM, Pita JC, Vigersky RA, Wilson YA, Loriaux DL (1978) Interaction of digitalis and spironolactone with human sex steroid receptors. *J Clin Endocrinol Metab* **46**: 338–344.

Rosenbaum PR, Rubin DP (1984) Reducing bias in observational studies using subclassification on the propensity score. *J Am Stat Assoc* **79**: 516–524.

WHO (2015) WHO Collaborating Centre for Drug Statistics Methodology. ATC/DDD Index 2015 (updated 9 Dec 2013; cited 9 Dec 2015). Available from http://www.whocc.no/atc_ddd_index/.

Wright JL, Hansten PD, Stanford JL (2014) Is digoxin use for cardiovascular disease associated with risk of prostate cancer? *Prostate* **74**: 97–102.

Zhang H, Qian DZ, Tan YS, Lee K, Gao P, Ren YR, Rey S, Hammers H, Chang D, Pili R, Dang CV, Liu JO, Semenza GL (2008) Digoxin and other cardiac glycosides inhibit HIF-1alpha synthesis and block tumor growth. *Proc Natl Acad Sci USA* **105**: 19579–19586.

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PUBLICATION IV

Cancer mortality does not differ by antiarrhythmic drug use: A population-based cohort of Finnish men

Kaapu KJ, Rantaniemi L, Talala K, Taari K, Tammela TLJ, Auvinen A, Murtola TJ

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SCIENTIFIC REPORTS

OPEN

Cancer mortality does not differ by antiarrhythmic drug use: A population-based cohort of Finnish men

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In-vitro **studies have suggested that the antiarrhythmic drug digoxin might restrain the growth of cancer cells by inhibiting Na+/K+-ATPase. We evaluated the association between cancer mortality and digoxin, sotalol and general antiarrhythmic drug use in a retrospective cohort study. The study population consists of 78,615 men originally identified for the Finnish Randomized Study of Screening for Prostate Cancer. Information on antiarrhythmic drug purchases was collected from the national prescription database. We used the Cox regression method to analyze separately overall cancer mortality and mortality from the most common types of cancer. During the median follow-up of 17.0 years after the baseline 28,936 (36.8%) men died, of these 8,889 due to cancer. 9,023 men (11.5%) had used antiarrhythmic drugs. Overall cancer mortality was elevated among antiarrhythmic drug users compared to non-users (HR 1.43, 95% CI 1.34–1.53). Similar results were observed separately for digoxin and for sotalol. However, the risk associations disappeared in long-term use and were modified by background co-morbidities. All in all, cancer mortality was elevated among antiarrhythmic drug users. This association is probably non-causal as it was related to short-term use and disappeared in long-term use. Our results do not support the anticancer effects of digoxin or any other antiarrhythmic drug.**

Various preclinical studies have suggested that the antiarrhythmic drug digoxin may have antineoplastic effects[1–3]. Digoxin may be able to inhibit growth of lung[4–6], prostate[7,8] and pancreatic[9] tumor cell lines and suppress cancer progression. The anticancer effects have been suggested to be due to inhibition of the plasma membrane Na^+/K^+-ATPase which increases intracellular concentration of Ca^{2+}, eventually causing apoptosis[7,10]. Another proposed mechanism is inhibition of HIF-1alpha, an important regulator of cell growth[8,11].

Digoxin use might be associated with a decreased risk of prostate cancer[12], especially among patients under regular PSA-surveillance[13,14], but is not associated with prostate cancer-specific survival[15–17]. In a British cohort study there was no association between digoxin use and cancer-specific survival from prostate, breast, respiratory or gastrointestinal cancer[18]. Further, digoxin use was not associated with survival among ovarian cancer patients[19].

Digoxin has estrogenic effects[20] and has been associated with an increased risk of breast[21] and uterine cancer but digoxin users may have a better prognosis and a decreased risk of metastases[22–24]. Digoxin use has also been linked to a higher risk of colorectal cancer[25] but no difference in cancer-specific survival after diagnosis of colorectal cancer was found in a population-based cohort study[26].

Studies concerning other antiarrhythmic drugs and cancer survival are sparse. The beta-blocker sotalol is both a K^+-channel blocker and used clinically as an antiarrhythmic drug. Adrenergic activation is essential for cancer and therefore the use of beta-blockers might be beneficial[27]. We have previously shown in a population-based

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	Antiarrhythmic drug use			Digoxin use			Sotalol use		
	Never	Ever	P-value	Never	Ever	P-value	Never	Ever	P-value
Characteristics of Participants									
Number of participants	69,592	9,023		72,286	6,329		76,311	2,304	
Median Age (IQR)	59 (55–63)	63 (59–67)	0.00	59 (55–63)	63 (59–67)	0.00	59 (55–63)	63 (59–67)	0.00
Median BMI (IQR)	26.3 (24.2–28.7)	27.2 (24.8–30.3)	0.00	26.3 (24.2–28.7)	27.4 (25.1–30.9)	0.00	26.3 (24.2–29.0)	27.2 (25.0–30.2)	0.00
Baseline cancer diagnosis (any)	2,822 (4.1%)	457 (5.1%)	0.00	2,956 (4.1%)	323 (5.1%)	0.00	3,165 (4.1%)	114 (4.9%)	0.06
Charlson comorbidity index			0.00			0.00			0.00
0	50,305 (72.3%)	4,703 (52.1%)		52,097 (72.1%)	2,911 (46.0%)		53,653 (70.3%)	1,355 (58.8%)	
1	3,192 (4.6%)	614 (6.8%)		3,322 (4.6%)	484 (7.6%)		3,683 (4.8%)	123 (5.3%)	
2 or greater	16,095 (23.1%)	3,706 (41.1%)		16,867 (23.3%)	2,934 (46.4%)		18,975 (24.9%)	826 (35.9%)	
Cancer death									
Overall cancer death	7,873 (11.3%)	1,016 (11.3%)		8,143 (11.3%)	746 (11.8%)		8,622 (11.3%)	267 (11.6%)	
Lung cancer death	2,090 (3.0%)	294 (3.3%)		2,152 (3.0%)	232 (3.7%)		2,320 (3.0%)	64 (2.8%)	
Colorectal cancer death	770 (1.1%)	91 (1.0%)		792 (1.1%)	69 (1.1%)		846 (1.1%)	15 (0.7%)	
Pancreatic cancer death	714 (1.0%)	68 (0.8%)		734 (1.0%)	48 (0.8%)		762 (1.0%)	20 (0.9%)	
Gastric cancer death	316 (0.5%)	27 (0.3%)		321 (0.4%)	22 (0.3%)		336 (0.4%)	7 (0.3%)	
Hepatic cancer	425 (0.6%)	48 (0.5%)		436 (0.6%)	37 (0.6%)		454 (0.6%)	19 (0.8%)	
Renal cancer	251 (0.4%)	35 (0.4%)		259 (0.4%)	27 (0.4%)		277 (0.4%)	9 (0.4%)	
Non-Hodgkin lymphoma	256 (0.4%)	46 (0.5%)		267 (0.4%)	35 (0.6%)		295 (0.4%)	7 (0.3%)	
Bladder cancer	190 (0.3%)	29 (0.3%)		198 (0.3%)	21 (0.3%)		215 (0.3%)	4 (0.2%)	
Central nervous system cancer	191 (0.3%)	17 (0.2%)		198 (0.3%)	10 (0.2%)		203 (0.3%)	5 (0.2%)	
Prevalence of medication use									
NSAIDs	54,837 (78.8%)	7,436 (82.5%)	0.00	57,145 (79.1%)	5,128 (81.0%)	0.00	60,311 (79.0%)	1,962 (85.2%)	0.00
Aspirin	10,732 (15.4%)	1,647 (18.3%)	0.00	11,287 (15.6%)	1092 (17.3%)	0.00	11,894 (15.6%)	485 (21.1%)	0.00
Statins	28,014 (40.3%)	4,840 (53.6%)	0.00	29,540 (40.9%)	3,314 (52.4%)	0.00	31,489 (41.3%)	1,374 (59.6%)	0.00
Antidiabetic drugs	13,321 (19.1%)	2,572 (28.5%)	0.00	13,871 (19.2%)	2,022 (31.9%)	0.00	15,274 (20.0%)	619 (26.8%)	0.00
Antihypertensives	44,472 (63.9%)	8,459 (93.7%)	0.00	46,878 (64.9%)	6,053 (95.6%)	0.00	50,731 (66.5%)	2,200 (95.5%)	0.00
Alpha-blockers	18,442 (26.5%)	2,901 (32.2%)	0.00	19,399 (26.8%)	1,944 (30.7%)	0.00	20,554 (26.9%)	789 (34.2%)	0.00

Table 1. Population characteristics in the Finnish Randomized Study of Screening for Prostate Cancer.

case-control study that sotalol is associated with a lowered prostate cancer risk[28] but does not associate with survival[17]. Beta-blockers as a group have been linked with prolonged cancer survival[29].

We estimated the association between use of digoxin, sotalol or other antiarrhythmic drugs and overall cancer mortality and separately lung, colorectal, pancreatic, liver, bladder, renal and CNS cancer mortality in a population-based cohort of Finnish men.

Results

Population characteristics. A total of 78,615 men with data from the SII prescription database were included in the study. Of these 9,023 (11.5%) had used at least one antiarrhythmic drug during the follow-up; 6,329 (8.1%) had used digoxin and 2,304 (2.9%) had used sotalol. The median age at baseline was 59 years among the never-users of antiarrhythmic drugs and 63 years among men with any antiarrhythmic drug use during the follow-up.

During the median follow-up of 17.0 years after baseline, 28,936 (36.8%) men died. There were 8,889 cancer deaths altogether, and the most frequent individual cancers were lung cancer (2,384 deaths), colorectal cancer (861 deaths) and pancreatic cancer (782 deaths) (Table 1).

In general, the use of other drugs (NSAIDs, aspirin, statins, antidiabetic drugs, antihypertensive drugs, alpha-blockers and 5-alpha-reductase inhibitors) was more common and the Charlson comorbidity index (CCI) was higher among antiarrhythmic drug users compared to non-users (Table 1).

Antiarrhythmic drug use and overall cancer mortality. Antiarrhythmic drug use in general was associated with increased cancer mortality in both age-adjusted and multivariable-adjusted analyses (multivariable-adjusted HR 1.43, 95% CI 1.34–1.53,). A similar risk increase was observed for men with digoxin use (HR 1.59, 95% CI 1.47–1.72) and sotalol use (HR 1.16, 95% CI 1.03–1.31) (Table 2). The risk increase attenuated with increasing amount, duration and intensity of drug use but there was no risk decrease even in long-term use (Table 3). Furthermore, the risk elevation tended to decrease also in lagged analysis estimating long-term effects of antiarrhythmic drug use (Table 2).

Antiarrhythmic drug use	Overall cancer death[a]		Lung cancer death	Colorectal cancer death	Pancreatic cancer death
	Age-adjusted model	Multivariable-adjusted model[b]	Multivariable-adjusted model[c]	Multivariable-adjusted model[c]	Multivariable-adjusted model[c]
	HR (95%CI)	HR (95%CI)	HR (95%CI)	HR (95%CI)	HR (95%CI)
No use	Ref	Ref	Ref	Ref	Ref
Any use	1.40 (1.31–1.50)	1.43 (1.34–1.53)	1.72 (1.52–1.95)	1.38 (1.11–1.73)	1.02 (0.79–1.31)
Lag 3 y	1.24 (1.15–1.34)	1.26 (1.17–1.36)	1.39 (1.20–1.61)	1.36 (1.07–1.74)	0.98 (0.74–1.30)
Lag 5 y	1.21 (1.12–1.31)	1.23 (1.13–1.33)	1.29 (1.10–1.51)	1.42 (1.10–1.82)	0.99 (0.74–1.33)
Digoxin use					
No use	Ref	Ref	Ref	Ref	Ref
Any use	1.60 (1.48–1.73)	1.59 (1.47–1.72)	2.10 (1.82–2.41)	1.59 (1.24–2.05)	1.06 (0.79–1.43)
Lag 3 y	1.35 (1.23–1.47)	1.33 (1.21–1.45)	1.59 (1.34–1.88)	1.53 (1.15–2.02)	1.00 (0.72–1.40)
Lag 5 y	1.30 (1.18–1.44)	1.28 (1.16–1.41)	1.49 (1.23–1.79)	1.59 (1.19–2.14)	0.97 (0.67–1.39)
Sotalol use					
No use	Ref	Ref	Ref	Ref	Ref
Any use	1.11 (0.98–1.25)	1.16 (1.03–1.31)	1.10 (0.85–1.41)	0.70 (0.42–1.17)	0.99 (0.63–1.54)
Lag 3 y	1.11 (0.98–1.26)	1.16 (1.02–1.32)	1.07 (0.82–1.39)	0.83 (0.51–1.37)	0.98 (0.61–1.57)
Lag 5 y	1.08 (0.95–1.24)	1.14 (0.99–1.30)	0.98 (0.74–1.31)	0.89 (0.54–1.47)	1.06 (0.66–1.69)

Table 2. Antiarrhythmic drug use and cancer mortality in Finnish Randomized Study of Screening for Prostate Cancer. [a]Including lung, prostate, colorectal, pancreatic, gastric, liver, renal, non-Hodgkin lymphoma, bladder and central nervous system cancer. [b]From Cox regression model adjusted for age, screening trial arm and use of cholesterol-lowering, antidiabetic and antihypertensive drugs, aspirin and other NSAIDs, 5alpha-reductase inhibitors, alpha-blockers and cancer diagnose at baseline. [c]From Cox regression model adjusted for age and use of cholesterol-lowering, antidiabetic and antihypertensive drugs, aspirin and other NSAIDs, 5alpha-reductase inhibitors, alpha-blockers and cancer diagnose at baseline.

Antiarrhythmic drug use and individual cancers. Both usage of antiarrhythmic drugs in general and usage of digoxin were associated with increased lung cancer mortality (HR 1.72, 95% CI 1.52–1.95 and HR 2.10, 95% CI 1.82–2.41, respectively). This association was not observed for sotalol use (HR 1.10, 95% CI 0.85–1.41) (Table 2). There were similar trends by amount, duration and intensity as with overall cancer mortality (Table 3).

The results for colorectal cancer mortality were rather similar to those for lung cancer mortality; Antiarrhythmic drug use in general and digoxin use both elevated risk of death (HR 1.38, 95% CI 1.11–1.73 and HR 1.59, 95% CI 1.24–2.05). Usage of sotalol was not associated with the risk for colorectal cancer death (HR 0.70, 95% CI 0.42–1.17) (Table 2).

Pancreatic cancer differed from other cancer types since antiarrhythmic drug use had no influence on pancreatic cancer mortality (HR 1.02, 95% CI 0.79–1.31). Identical findings were observed for digoxin use (HR 1.06, 95% CI 0.79–1.43) and for sotalol use (HR 0.99, 95% CI 0.63–1.54).

Furthermore, antiarrhythmic drug use and digoxin use were associated with elevated risk of death due to non-Hodgkin lymphoma and bladder cancer (Table S1).

Subgroup analysis. The overall cancer mortality of antiarrhythmic drug users was increased in all subgroups that we analyzed (Fig. 1). The risk estimates for overall cancer death were most increased among non-users of antihypertensive drugs (p for interaction 0.01). There was a similar risk difference between users and non-users of antihypertensive drugs among digoxin users (p for interaction 0.002). Furthermore, there was an interaction by antidiabetic drug use, the risk being higher among men who were not using antidiabetic drugs (p for interaction 0.01) (Fig. 1).

We used the CCI to stratify the study population by comorbidities. Antiarrhythmic drug use associated with increased cancer mortality among the men with least comorbidities (Charlson index 0: HR 1.37, 95% CI 1.19–1.56). A similar result was observed among men with intermediate comorbidities (Charlson index 1: HR 1.22, 95% CI 0.87–1.71) but the CIs are wider since there were less men in this cohort. There was no association between cancer mortality and antiarrhythmic drug use among men with the most comorbidities (Charlson index 2 or greater: 0.98, 95% CI 0.91–1.06). There was a statistically significant effect modification by CCI (p for interaction < 0.001).

Sensitivity analysis. To evaluate confounding by indication we estimated the risk association between the indications for antiarrhythmic drug and digoxin use (cardiac insufficiency and arrhythmias) and cancer mortality. 4,199 men had recorded diagnosis of cardiac insufficiency (ICD-10 codes I50) in the HILMO database, while 1,507 men had a diagnosis of arrhythmia (I47 and I49). The increase in overall cancer mortality risk that we observed for cardiac insufficiency (HR 1.19, 95% CI 1.08–1.31) was similar to the risk for antiarrhythmic drug use in general that we observed in our main analysis. However, having a recorded diagnosis of arrhythmia was associated with a lowered risk of cancer death (HR 0.76, 95% CI 0.64–0.90). There was no association between antiarrhythmic drug use and cancer mortality in competing risk analyses. The HR for overall antiarrhythmic drug

	All antiarrhythmic drugs			Digoxin			Sotalol		
	Overall cancer mortality	Lung cancer mortality	Pancreatic cancer mortality	Overall cancer mortality	Lung cancer mortality	Pancreatic cancer mortality	Overall cancer mortality	Lung cancer mortality	Pancreatic cancer mortality
	HR (95% CI)[a]	HR (95% CI)[a]	HR (95% CI)[a]	HR (95% CI)[a]	HR (95% CI)[a]	HR (95% CI)[a]	HR (95% CI)[a]	HR (95% CI)[a]	HR (95% CI)[a]
Cumulative quantity of medication use[b]									
DDD tertiles									
1st tertile	1.85 (1.67–2.05)	2.22 (1.84–2.67)	1.30 (0.87–1.92)	1.97 (1.76–2.21)	2.47 (2.00–3.04)	1.31 (0.83–2.07)	1.18 (0.97–1.44)	1.29 (0.88–1.88)	0.84 (0.38–1.88)
2nd tertile	1.39 (1.25–1.55)	1.88 (1.56–2.28)	0.95 (0.62–1.45)	1.59 (1.39–1.81)	2.03 (1.59–2.58)	1.14 (0.69–1.88)	1.15 (0.93–1.43)	1.17 (0.77–1.76)	1.07 (0.51–2.25)
3rd tertile	1.10 (0.97–1.25)	1.07 (0.83–1.38)	0.84 (0.54–1.32)	1.22 (1.06–1.41)	1.77 (1.39–2.27)	0.78 (0.45–1.35)	1.14 (0.92–1.42)	0.79 (0.47–1.34)	1.07 (0.51–2.26)
Duration of medication use[c]									
Year tertiles									
1st tertile	1.72 (1.56–1.89)	2.14 (1.80–2.55)	1.14 (0.78–1.67)	1.84 (1.66–2.05)	2.46 (2.04–2.96)	1.35 (0.90–2.02)	1.32 (1.09–1.60)	1.53 (1.07–2.18)	0.72 (0.30–1.74)
2nd tertile	1.36 (1.22–1.51)	1.72 (1.41–2.09)	0.77 (0.49–1.23)	1.61 (1.41–1.84)	2.18 (1.72–2.76)	0.86 (0.48–1.52)	1.00 (0.81–1.22)	0.88 (0.57–1.35)	0.74 (0.33–1.65)
3rd tertile	1.13 (0.98–1.30)	1.06 (0.79–1.43)	1.17 (0.75–1.82)	1.17 (0.99–1.38)	1.37 (0.99–1.88)	0.86 (0.47–1.56)	1.21 (0.95–1.53)	0.84 (0.48–1.49)	1.75 (0.90–3.38)
Intensity of medication use (DDDs/year)[d]									
Intensity tertiles									
1st tertile	1.91 (1.72–2.11)	2.26 (1.87–2.74)	1.25 (0.83–1.89)	2.13 (1.91–2.38)	2.71 (2.22–3.30)	1.30 (0.82–2.05)	1.19 (0.97–1.46)	1.25 (0.85–1.86)	1.18 (0.59–2.36)
2nd tertile	1.42 (1.26–1.59)	1.75 (1.41–2.16)	1.08 (0.71–1.66)	1.49 (1.28–1.74)	1.93 (1.46–2.56)	0.91 (0.49–1.71)	1.22 (0.99–1.50)	1.04 (0.67–1.61)	1.10 (0.52–2.32)
3rd tertile	1.10 (0.98–1.24)	1.30 (1.05–1.61)	0.80 (0.52–1.23)	1.20 (1.06–1.37)	1.68 (1.33–2.11)	0.97 (0.61–1.53)	1.08 (0.87–1.34)	0.99 (0.63–1.56)	0.71 (0.29–1.71)

Table 3. Cancer mortality by amount, duration and intensity of antiarrhythmic drug use in the the Finnish Randomized Study of Screening for Prostate Cancer. [a]From Cox regression model adjusted for age, screening trial arm (only for overall cancer mortality) and use of cholesterol-lowering, antidiabetic and antihypertensive drugs, aspirin and other NSAIDs, and 5alpha-reductase inhibitors and alpha-blockers. [b]Tertile cut-points for cumulative amount of medication use: All antiarrhythmic drugs combined 1st tertile: 1–280 DDD, 2nd tertile: 281–1,400 DDD, 3rd tertile: more than 1,400 DDD; Digoxin 1st tertile: 1–200 DDD, 2nd tertile: 201–960 DDD, 3rd tertile: more than 960 DDD; Sotalol 1st tertile: 1–200 DDD, 2nd tertile: 201–1,230 DDD, 3rd tertile: more than 1,230 DDD. [c]Tertile cut-points for cumulative duration of medication use: All antiarrhythmic drugs combined 1st tertile: 1–2 years, 2nd tertile: 3–7 years, 3rd tertile: longer than 7 years; Digoxin 1st tertile: 1–2 years, 2nd tertile: 3–6 years, 3rd tertile: longer than 6 years; Sotalol 1st tertile: 1 year, 2nd tertile: 2–5 years, 3rd tertile: longer than 5 years. [d]Tertile cut-points for intensity of medication use: All antiarrhythmic drugs combined 1st tertile: 1–116 DDDs/year, 2nd tertile: 117–228 DDDs/year, 3rd tertile: more than 229 DDDs/year; Digoxin 1st tertile: 1–100 DDDs/year, 2nd tertile: 101–170 DDDs/year, 3rd tertile: more than 170 DDDs/year; Sotalol 1st tertile: 1–120 DDDs/year, 2nd tertile: 121–285 DDDs/year, 3rd tertile: more than 285 DDDs/year.

use was 1.04 (95% CI 0.97–1.12). For digoxin and sotalol users the HRs were 1.01 (95% CI 0.93–1.10) and 1.03 (95% CI 0.91–1.17), respectively.

Both overall antiarrhythmic drug use (HR 1.13, 95% CI 1.05–1.21) and digoxin use (HR 1.13, 95% CI 1.05–1.23) were associated with increased cancer mortality in a sensitivity analysis adjusted by the CCI. However, the risk estimates were lower compared to the main analyses. In this analysis, sotalol use had no effect on cancer mortality (HR 0.98, 95% CI 0.86–1.10). In addition, the CCI was independently associated with an increased risk of cancer death; HR 1.51, 95% CI 1.50–1.52 per increase of one point.

All-cause mortality among antiarrhythmic drug users was increased compared to non-users (HR 2.14, 95% CI 2.07–2.21). Digoxin users had an even greater risk of death (HR 2.52, 95% CI 2.43–2.61), whereas sotalol users had a minor, but nevertheless statistically significant, increase in mortality (HR 1.35, 95% CI 1.27–1.44). Excluding prevalent cancers at baseline from analysis did not modify results (Table S2).

Compared to the users of other antiarrhythmic drugs, digoxin users had an increased risk of cancer death (HR 3.06, 95% CI 2.64–3.54). Sotalol use was not associated with cancer mortality (HR 1.12, 95% CI 0.99–1.32 in a similar sensitivity analysis.

Discussion

The usage of antiarrhythmic drugs was associated with elevated overall cancer mortality and with increased lung cancer mortality in this retrospective cohort study. Digoxin users had a more prominent increase in risk estimates for cancer death, compared to overall antiarrhythmic drug users. The individual cancer types with increased mortality by digoxin use were lung cancer, colorectal cancer, bladder cancer and non-Hodgkin lymphoma. Usage of sotalol and cancer mortality had no association in the age-adjusted analysis but in the multivariable analysis users had a statistically significant increase in the risk of cancer-specific death.

Digoxin's mechanism of action differs from other classic antiarrhythmic drugs. Vaughan Williams classification is used to categorize antiarrhythmic agents by mechanism of action. Class I is divided to subclasses Ia, Ib and Ic, all of which are Na^+-channel blockers. Class II includes beta-blockers (excluding sotalol) and Class III K^+-channel blockers. Finally, Ca^{2+}-channel blockers form class IV and agents with unknown or other mechanisms form class V. Digoxin belongs to the Class V and is a Na^+/K^+-ATPase inhibitor. This increases intracellular Na^+-concentration leading to decreased activity of Na^+/Ca^{2+}-exchanger. Eventually, this cascade results in increased concentration of calcium-ions, which might induce apoptosis[7,10].

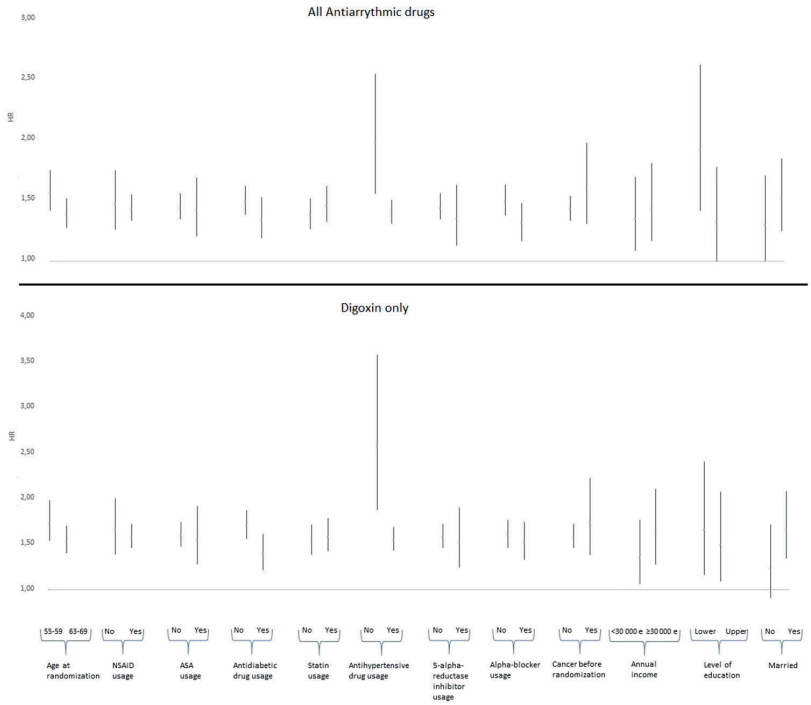

Figure 1. Overall cancer mortality by overall antiarrhythmic drug use and by digoxin use versus non-use stratified by patient characteristics in the the Finnish Randomized Study of Screening for Prostate Cancer.

Divergences between users and non-users (confounding by indication) provide a likely explanation for the observed increase in cancer mortality. When we analyzed the association between cardiac insufficiency (the indication) and cancer mortality, a comparable risk elevation was observed. Furthermore, the risk increase tended to disappear with increasing amount, duration and intensity of antiarrhythmic drug use, suggesting that the increased mortality is unlikely to be caused by antiarrhythmic drug use but rather by residual confounding by unmeasured background differences between antiarrhythmic drug uses and non-users. If the drugs did indeed increase the risk, an opposite trend would be presumed.

Digoxin users are likely more fragile than non-users, which may cause non-causal risk differences in epidemiological studies. This explanation was supported by subgroup analyses stratified by the CCI; among men with a low co-morbidity burden, digoxin use was associated with an increased risk of cancer death. However, among men with a high co-morbidity burden, the risk difference disappeared. This confirms that the risk association is modified by background co-morbidities. Further, the CCI was an independent risk factor for cancer death. In the competing risk analyses antiarrhythmic drug use was not associated with cancer mortality, further supporting the notion that use of digoxin or other antiarrhythmic drugs does not affect cancer mortality when non-cancer deaths are taken into account. When compared to users of other antiarrhythmic drugs, digoxin users had an increased cancer mortality. Therefore, the co-morbidity burden may differ even between users of different antiarrhythmic drugs.

Our main results are slightly inconsistent with previously published ones. There are no studies concerning overall cancer mortality and few studies about individual cancer types. *In vitro* studies have suggested that digoxin might have a suppressive effect on lung neoplasms via multiple mechanisms; it has been shown that digoxin hinders tumor progression by inhibiting the activation of an important oncogene Src[4]. Moreover, digoxin decreases the expression of VEGF and NDRG1 through inhibition of HIF-1alpha synthesis[5] and induces autophagy through the regulation of mTOR and ERK1/2 signaling pathways in non-small cell lung cancer cells[6]. A Swedish study observed that digoxin users had a diminished risk of lung neoplasms (HR 0.55, 95% CI 0.39–0.79) compared to users of organic nitrates[30]. Nonetheless, these chemopreventive features of digoxin did not translate into diminished lung cancer mortality in our large population-based study.

One population-based cohort study regarding colorectal cancer survival has previously been published[26]. The study included 10,357 patients with a colorectal cancer diagnosis and during the median follow-up of 4.8 years 2,724 colorectal cancer–specific deaths occurred. Before model adjustments digoxin use was associated with elevated colorectal cancer–specific mortality (HR 1.25, 95% CI 1.07–1.46), but the association disappeared after adjustment for confounders (HR 1.10, 95% CI 0.91–1.34). In our study, digoxin users had slightly elevated colorectal cancer mortality in the multivariable adjusted analysis. This inconsistency is probably due to differences

in adjustment-models. Karasneh *et al.*[26] were able to adjust the analysis for received radiotherapy, chemotherapy or surgery within 6 months and for comorbidities more comprehensively compared to us.

Interestingly, pancreatic cancer differed from other cancer types. There was no association between pancreatic cancer mortality and digoxin use, whereas there was a statistically significant risk increase for other major cancer types. It has been observed that there is an elevated level of HIF-1alpha expression in pancreatic cancer[31] and a previous study observed that intraperitoneal digoxin injections significantly reduced pancreatic tumor volume compared to placebo-injections[9]. Furthermore, the same study noticed that digoxin injections decreased the expression of stem cell factor (SCF), a cytokine commonly involved in tumor progression. However, these pathways should be relevant also for other cancer types besides pancreatic cancer. Thus, the differing risk association between digoxin use and pancreatic cancer could be due to other causes.

In contrast to other cancer types, there was no association between sotalol use and death due to lung and colorectal cancer. Sotalol is both a beta-blocker and K^+-channel blocker and it is possible that these properties may overcome the otherwise increased cancer mortality among antiarrhythmic drug users in these cancer types. Another possibility is that sotalol users had a different distribution of co-morbidities and therefore confounding by indication could have less of an effect. Furthermore, the number of sotalol users was lower compared to digoxin users, resulting in wider confidence intervals and less robust results.

Our study had several strengths. First, we had a large population-based cohort that comprehensively represents the Finnish male population. Additionally, detailed information on antiarrhythmic drug purchases was available, allowing us to calculate the individual amounts and durations of drug use. Consequently, we were able to perform time-dependent regression analyses in order to control for the immortal time bias. We were able to adjust for comorbidities and drugs regularly used along with antiarrhythmic drugs since the information was available from the national registries.

On the other hand, a few limitations should be discussed. There was no information on exact indications of antiarrhythmic drug use even though we were able to separately evaluate the most common indications. We also lacked data on lifestyle habits such as diet, alcohol consumption, smoking and physical activity, all of which may be risk factors for cancer death. Furthermore, there was no information on tumor grade, metastases or given treatment. Since we were not able to adjust analyses by these factors, confounding is possible. Smoking is a risk factor for both cardiac diseases and cancer death thus it could be a confounding factor increasing observed cancer mortality among antiarrhythmic drug users. We did not have information on frequency of health care contacts. This might have been greater among antiarrhythmic drug users and therefore leading to earlier detection of tumors, which might result in lower observed cancer mortality.

Our information on medication use is based on reimbursed drug purchases. We do not know for sure whether or not patients have actually used the drugs they have bought. Finally, the study population was originally recruited for a prostate cancer screening trial. The cohort included principally Caucasian men so there is no certainty whether the results can be generalized to women or other ethnic groups.

Conclusion

We observed that antiarrhythmic drug use has neither general cancer protective effects nor a beneficial impact on any particular cancer type. In contrast, cancer mortality was increased among antiarrhythmic drug users compared to non-users, but the risk increase was likely non-causal as it was related to short-term use only and disappeared in long-term use. Our results do not support the hypothesis of digoxin's anticancer effects or those of any other antiarrhythmic drug.

Material and Methods

Study cohort. We used the population of the Finnish Randomized Study of Screening for Prostate Cancer (FinRSPC), which is the largest component of the European Randomized Study of Screening for Prostate Cancer (ERSPC). The detailed trial protocol has been described previously[32]. In short, 80,458 men were recruited to the study during the years 1996–1999. Men were randomized to either the screening arm (31,866 men, prostate-specific antigen test at 4-year intervals) or control arm (48,278 men, no intervention, followed through national cancer registry). The follow-up continued until the end of 2015. Prevalent prostate cancer cases at baseline were excluded; no exclusions for other cancers were made.

The official causes of death in 1996–2015 were obtained from the death certificate registry of Statistics Finland. FinRSPC cause-of-death committee has previously found Statistics Finland to be a dependable source of data (kappa 0.95)[33]. The data included primary, immediate and contributory causes of death recorded as ICD-10 codes. For this study we collected information on deaths with lung (C34), colorectal (C18), pancreatic (C25), gastric (C16), liver (C22), renal (C64), non-Hodgkin lymphoma (C81), bladder (C67) or central nervous system cancer (C71 and C72) recorded as the primary cause of death. Prostate cancer deaths were included in overall cancer mortality. We have previously performed a separate analysis for the risk of prostate cancer death[17].

Information on diagnoses recorded during in- and outpatient hospital contacts during 1996–2012 were obtained from the Care Register for Health Care (HILMO) of the National Institute for Health and Welfare. The data was used to calculate the CCI for the study participants. Additionally, we sought information on indications for antiarrhythmic drug use: heart failure (ICD-10 code I50) and cardiac arrhythmias (I47 and I49).

The study was approved by the Ethics Committee of the Pirkanmaa Health Care District, Finland (tracking number R10167) and the Committee confirmed that all research was performed in accordance with relevant guidelines. Informed consent was obtained from all participants in the screening arm of the study.

Information on medication use. We collected data on antiarrhythmic drug purchases during 1995–2015 from the reimbursement database of the Social Insurance Institution of Finland (SII). SII is a governmental

agency that provides reimbursements for physician-prescribed drug purchases to all Finnish citizens as part of a national health insurance. All reimbursed purchases are registered in the database that records the date, number of packages acquired, and number and dosage of the tablets for every purchase. This information allowed us to calculate the amount of the medication purchases for each drug on a yearly basis.

Antiarrhythmic drug purchases were identified with drug-specific ATC–codes. Drugs in clinical use during the study period were amiodarone, digoxin, disopyramide, etilefrine, flecainide, quinidine, mexiletine, procainamide, propafenone and sotalol. Additionally, we obtained information on use of statins, antidiabetic medication (oral glucose-lowering drugs and insulins), antihypertensive medication (beta-blockers, ACE-inhibitors/ATII receptor blockers, calcium-channel blockers, diuretics and other types of drugs, such as methyldopa and clonidine), aspirin and other NSAIDs, 5-alpha-reductase inhibitors and alpha-blockers.

Statistical analysis. The baseline characteristics were compared between ever-users and never-users of antiarrhythmic drugs using the Chi-square test for categorical variables and the Mann-Whitney U test for continuous variables. The association between antiarrhythmic drug use and cancer mortality was estimated using the Cox proportional hazards regression. We estimated hazard ratios (HR) and their 95% confidence intervals (CIs) for cancer death overall and for deaths due to specific types of cancer by antiarrhythmic drug use. We analyzed mortality by overall antiarrhythmic drug use and separately by digoxin and sotalol use. The follow-up time was calculated from FinRSPC randomization to the date of death, emigration or the common closing date (December 31th 2015), whichever occurred first.

Antiarrhythmic drug use was analyzed as a time-dependent variable to minimize immortal time bias. Therefore, we updated the medication use status prospectively for every year of follow-up by annual medication purchases. If there was a recorded purchase at any point during a year, the man was regarded as a user. If medication purchases were stopped during the follow-up, the participant remained in the user category to minimize bias due to selective discontinuation of medication use in the terminal phase of cancer. Men without any purchases during the follow-up and all users before the first purchase were classified as non-users, which was used as the reference group in the main analyses. Age-adjusted and multivariable analyses (further adjustment for baseline cancer diagnosis and use of other drug groups: drugs used in management of benign prostatic hyperplasia, diabetes, hypercholesterolemia or hypertension, and aspirin and other NSAIDs) were conducted. Besides antiarrhythmic drugs, use of other drugs was included in the analyses as a time-independent variable.

We standardized amounts of antiarrhythmic drugs use by dividing the cumulative annual milligram amount of each drug with the standard Defined Daily Dose (DDD) published on the WHO website[34]. By adding together years with antiarrhythmic drug purchases, we were able to estimate cumulative duration of drug use. Intensity of drug use (DDDs/year) was calculated by dividing the cumulative annual amount with duration of medication use. We stratified men into tertiles by the variables mentioned above to estimate whether the amount or duration of drug use affects mortality.

Effect modification by age, baseline cancer, use of other drug groups and socioeconomic factors was evaluated in subgroup analyses stratified according to these variables. The statistical significance of each effect modifier was evaluated by adding an interaction term between antiarrhythmic drug use and the background variable into the multivariable-adjusted Cox regression model.

We evaluated the long-term effects of antiarrhythmic drug use in lag-time analyses, in which the time-dependent status of antiarrhythmic drug use was lagged forward 3–5 years in follow-up time. These analyses were carried out to minimize confounding by indication, as especially digoxin is commonly used in management of potentially lethal congestive heart failure. In addition, we performed competing risk regression analyses with non-cancer deaths as the competing risk. These analyses were conducted according to the method reported by Fine and Gray[35].

The statistical tests were two-sided. P-values of 0.05 or less were considered statistically significant. IBM SPSS Statistics 23 (Chicago, IL, USA) software was used for data analyses.

References

1. Menger, L. *et al*. Cardiac glycosides exert anticancer effects by inducing immunogenic cell death. *Sci. Transl. Med.* **4**, 143 (2012).
2. Lu, G. Y., Liu, S. T., Huang, S. M., Chang, Y. L. & Lin, W. S. Multiple effects of digoxin on subsets of cancer-associated genes through the alternative splicing pathway. *Biochimie.* **106**, 131–139 (2014).
3. Rocha, S. C. *et al*. 21-Benzylidene digoxin: a proapoptotic cardenolide of cancer cells that up-regulates Na,K-ATPase and epithelial tight junctions. *PLoS ONE* **9**, e108776 (2014).
4. Lin, S. Y. *et al*. Digoxin Suppresses Tumor Malignancy through Inhibiting Multiple Src-Related Signaling Pathways in Non-Small Cell Lung Cancer. *PLoS ONE* **10**, e0123305 (2015).
5. Wei, D. *et al*. Digoxin downregulates NDRG1 and VEGF through the inhibition of HIF-1alpha under hypoxic conditions in human lung adenocarcinoma A549 cells. *Int. J. Mol. Sci.* **14**, 7273–7285 (2013).
6. Wang, Y. *et al*. Cardiac glycosides induce autophagy in human non-small cell lung cancer cells through regulation of dual signaling pathways. *Int. J. Biochem. Cell Biol.* **44**, 1813–1824 (2012).
7. McConkey, D. J., Lin, Y., Nutt, L. K., Ozel, H. Z. & Newman, R. A. Cardiac glycosides stimulate Ca2+ increases and apoptosis in androgen-independent, metastatic human prostate adenocarcinoma cells. *Cancer Res.* **60**, 3807–3812 (2010).
8. Gayed, B. A., O'Malley, K. J., Pilch, J. & Wang, Z. Digoxin inhibits blood vessel density and HIF-1a expression in castration-resistant C4-2 xenograft prostate tumors. *Clin. Transl. Sci.* **5**, 39–42 (2012).
9. Gao, C. *et al*. SCF, regulated by HIF-1alpha, promotes pancreatic ductal adenocarcinoma cell progression. *PLoS ONE* **10**, e0121338 (2015).
10. Prevarskaya, N., Skryma, R. & Shuba, Y. Ca2+ homeostasis in apoptotic resistance of prostate cancer cells. *Biochem. Biophys. Res. Commun.* **322**, 1326–1335 (2004).
11. Lewis, J. A. Digoxin blocks tumor growth through HIF-1alpha inhibition. *Curr. Top. Med. Chem.* **9**, 117 (2009).
12. Platz, E. A. *et al*. A novel two-stage, transdisciplinary study identifies digoxin as a possible drug for prostate cancer treatment. *Cancer Discov.* **1**, 68–77 (2011).

13. Kaapu, K. J. *et al*. Prostate cancer risk among users of digoxin and other antiarrhythmic drugs in the Finnish Prostate Cancer Screening Trial. *Cancer Causes Control* **27**, 157–164 (2016).
14. Wright, J. L., Hansten, P. D. & Stanford, J. L. Is digoxin use for cardiovascular disease associated with risk of prostate cancer? *Prostate* **74**, 97–102 (2013).
15. Flahavan, E. M., Sharp, L., Bennett, K. & Barron, T. I. A cohort study of digoxin exposure and mortality in men with prostate cancer. *BJU Int.* **113**, 236–245 (2014).
16. Karasneh, R. A., Murray, L. J., Hughes, C. M. & Cardwell, C. R. Digoxin use after diagnosis of prostate cancer and survival: a population-based cohort study. *Pharmacoepidemiol. Drug Saf.* **25**, 1099–1103 (2016).
17. Kaapu, K. J. *et al*. Digoxin and prostate cancer survival in the Finnish Randomized Study of Screening for Prostate Cancer. *Br. J. Cancer* **115**, 1289–1295 (2016).
18. Lip, S. *et al*. Incidence and prognosis of cancer associated with digoxin and common antihypertensive drugs. *J. Hypertens.* **33**(Suppl 1), E45 (2015).
19. Vogel, T. J., Jeon, C., Karlan, B. & Walsh, C. Digoxin therapy is not associated with improved survival in epithelial ovarian cancer: A SEER-Medicare database analysis. *Gynecol. Oncol.* **140**, 285–288 (2016).
20. Rifka, S. M., Pita, J. C., Vigersky, R. A., Wilson, Y. A. & Loriaux, D. L. Interaction of digitalis and spironolactone with human sex steroid receptors. *J. Clin. Endocrinol. Metab.* **46**, 338–344 (1978).
21. Ahern, T. P., Tamimi, R. M., Rosner, B. A. & Hankinson, S. E. Digoxin use and risk of invasive breast cancer: evidence from the Nurses' Health Study and meta-analysis. *Breast Cancer Res. Treat.* **144**, 427–435 (2014).
22. Biggar, R. J., Andersen, E. W., Kroman, N., Wohlfahrt, J. & Melbye, M. Breast cancer in women using digoxin: tumor characteristics and relapse risk. *Breast Cancer Res.* **15**, R13 (2013).
23. Wong, C. C. *et al*. Inhibitors of hypoxia-inducible factor 1 block breast cancer metastatic niche formation and lung metastasis. *J. Mol. Med.* **90**, 803–815 (2013).
24. Karasneh, R. A., Murray, L. J., Mc Menamin, U. C., Hughes, C. M. & Cardwell, C. R. Digoxin use after diagnosis of breast cancer and survival: a population-based cohort study. *Breast Cancer Res. Treat.* **151**, 661–669 (2015).
25. Boursi, B., Haynes, K., Mamtani, R. & Yang, Y. X. Digoxin use and the risk for colorectal cancer. *Pharmacoepidemiol. Drug Saf.* **23**, 1147–1153 (2014).
26. Karasneh, R. A., Murray, L. J., Hughes, C. M. & Cardwell, C. R. Digoxin use after diagnosis of colorectal cancer and survival: a population-based cohort study. *Cancer Epidemiol. Biomarkers Prev.* **24**, 1804–1807 (2015).
27. Cole, S. W. & Sood, A. K. Molecular pathways: beta-adrenergic signaling in cancer. *Clin. Cancer Res.* **18**, 1201–1206 (2016).
28. Kaapu, K. J., Ahti, J., Tammela, T. L., Auvinen, A. & Murtola, T. J. Sotalol, but not digoxin is associated with decreased prostate cancer risk: A population-based case-control study. *Int. J. Cancer* **137**, 1187–1195 (2015).
29. Choi, C. H. *et al*. Meta-analysis of the effects of beta blocker on survival time in cancer patients. *J. Cancer Res. Clin. Oncol.* **140**, 1179–1188 (2014).
30. Li, W., Xie, S. H., Tse, L. A. & Lagergren, J. Digitalis use and lung cancer risk by histological type in men. *Int. J. Cancer* **141**, 1981–1986 (2017).
31. Miyake, K. *et al*. The novel hypoxic cytotoxin, TX-2098 has antitumor effect in pancreatic cancer; possible mechanism through inhibiting VEGF and hypoxia inducible factor-1alpha targeted gene expression. *Exp. Cell Res.* **318**, 1554–1563 (2012).
32. Kilpeläinen, T. P. *et al*. Prostate Cancer Mortality in the Finnish Randomized Screening Trial. *J. Natl. Cancer Inst.* **105**, 719–725 (2013).
33. Mäkinen, T. *et al*. Assessment of causes of death in a prostate cancer screening trial. *Int. J. Cancer* **122**, 413–417 (2008).
34. WHO Collaborating Centre for Drug Statistics Methodology. ATC/DDD Index 2017. [updated 2016 Dec 19th; cited 2017 Mar 12th] Available from: http://www.whocc.no/atc_ddd_index/.
35. Fine, J. P. & Gray, R. J. A proportional hazards model for the subdistribution of a competing risk. *J. Am. Stat. Assoc.* **94**, 496–509 (1999).

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Author Contributions

K. Taa, T.L.J.T and A.A. designed the research; K. Tal. maintains and updates the data; K.J.K. and L.R. analyzed the data; K.J.K. and T.J.M. wrote the manuscript and prepared the tables and the figures. All authors reviewed and commented on the manuscript at all stages.

Additional Information

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